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(54) Title: MYOSIN IXa AND CYCLIC NUCLEOTIDE GATED CHANNEL–15 (CNGC–15) POLYNUCLEOTIDES, POLYPEPTIDES, COMPOSITIONS, METHODS AND USES THEREOF

(57) Abstract

The present invention discloses the amino acid and nucleic acid sequences of a new CNGC and Myosin that map to the region of the human chromosome associated with Bardet–Biedl Syndrome. Cyclic nucleotide gated channels (CNGCs) comprise a family of multimeric protein ion channels that open in response to the binding of a cyclic nucleotide to an intracellular domain. The two new proteins, CNGC–15 and Myosin IXa, are useful in the study, diagnosis and treatment of Bardet–Biedl Syndrome and Usher Syndrome. Other indications that can be treated by CNGC–15 and/or Myosin IXa polypeptides, or agonists or antagonists include hearing loss, retinis pigmentosa, obesity, hypogonadism, sterility, polydactyly, brachydactyly, syndactyly, mental retardation, renal abnormalities, hypertension, diabetes and cardiovascular abnormalities. Compositions and methods for expressing cyclic nucleotide gates channel–15 (CNGC–15) and Myosin IXa are provided. The compositions comprise CNGC–15 and Myosin IXa polypeptides and derivatives thereof, nucleotide sequences, expression cassettes, transformed cells and antibodies to these polypeptides. Methods for the expression and detection of CNGC–15 and Myosin IXa nucleotides and polypeptides and compositions for the treatment of these conditions are provided.

MYOSIN IXa Polypeptide Sequence (SEQ ID NO:1)

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MPIHDGGRRRFRDKHTLRIYPCAISKGTITCPIEARRHSTAASVIKSLI   50
NKLLHLDRTKCYVLAEVKEFGGRSNILNPTDCPWQQMNLWFRMALERRLSG  100
EDTRFLLRERKLDGSIHYGSLQSNLRVTERERKRMMKRGFLPQFQQKDTDD  150
LCSLPDLNKEYLLEKLRDPKHRKTTTVGSILIVTNPFKPLPIYNPKIV  200
KHYDNRQLGKPEPHIYAVADVAYHAMLQRKENQCIVISGESGSGKTQSTN  250
FLIHHLTALSQKGFASCVEQIILGAGPVLRAFGNAKTARDNNSSRFGKFI  300
QVHYQRTGTVLGAYVEKYLLERSRLVTQSKDERSFHVFYYLLAGASEDER  350
SAPHLKQPKEYHYLNQSYTKKPLAQEEDDTCYDSPDCPTVEGKDLRSHDPE  400
MLQLAMEMVGFLPKYRRQIFSLLSAIIHLGNICYKEKTYRDDSIDICRPE  450
VLPIVSKLLEVKEELFAALVYRSTVTVPGRKLTLPTKLAEAVTVKHSNAR  500
SLYSALFDWIVFRINHALLRSKEDLERSFTSLGVDLVPCFEDYEKNWSFE  550
QFCIEFANERLQHYYRQHIFKLSQEEYTREGISWEHIDYIDNTCCIKLIS  600
KKPTGLLSLLDRESNFPQATWQTLLDKFKKQHKDNSYIKFPAVNKPAFII  650
KHYAGKVKYGVKDFREKNTDNKRPDIVALLRSSKNAFIQGNIGIDPVAVF  700
RHAILRAFFRAMVAFREAGKRSIHRKTGHDDTAPCAILKSHDSFSPLQHP  750
VKQRSLKILQRCKSEKYSITRKKPRTPLSDKQGKRALMKEKMQRDTFDIAN  800
NGRTGIRQSRLSSGTSLLDKDGIFANSTSSKLLKRASGILTPKKKFKSP  850
ALPKHLAEVHSLKHLTRLYLQDRITKSLLELHKKKKPPSISAQFQASLSK  900
LMETLGQAEPTYVKCIRSNAEKLPLRFSDVLVLRQLRYTGMLETVQIRQS  950
GYSSKYSPQDFVSHFRVLLPRNIIPSKFWIQDFFRRILMPDHTQWGKTH 1000
VFLKSQESQRLQDLLRQEVLRAIILLQSHFRVLLCRQEFLKLRQASVIIQ 1050
RYRNHTLRQEQVHRDAVQKDAFVWASAWALLQAHFRARLERQAYLELRAA 1100
AIVIQQKWRDYYRRRRMAAICIQARFRAYRESKAYQKQRKKILLQSTCR 1150
GFRARQRFKALKSGRLRKTKFSVGLVWIKEYGSLETQGSDPSERKDCSFD 1200
KRIKAIEECKSVIESRRISRESSVDCLKESPNKQGRRAQSQSGVDLQEDV 1250
LVRERPRSLEDLSQKVVGRAKRESRRGRELEQAIFSLELKVRELGGISP 1300
SEDRRNSTELVPEGLQSPAGTPDSESSQGSLELLSYKESQRSKLRSVISD 1350
EGDLQFPSPKISSSFKFDSRDWALSASNETSSASELKDGTHKEHVVCSSE 1400
SITCKPQLKDSFISSELPTFVFIPQQDFLKTKQLOTSIQRQKLLEHEDT 1450
AGEALTLDINRETRATHCSGKDQIVPSLFTESNFVLKKLKKLMTKKEER 1500
QKQLQQQHERKEHSEQIRQQTDILKKKHAAFKTIHKPRIGECLVAPSSTQS 1550
KQRVERPSSLLSLSTSWKGELMVLGSLSLKDAALAQKDSSSAELPFKDAP 1600
VTVFPEARGSPCQESTVKELSKYDRSGTQLAVACKLSHSRISKRERFRPT 1650
QSYSRRSDDLSEEGKAARPIFYTPKDWESIPLVSKEALASSKNPQLMKEDKF 1700
ANKPVKLAGPQQRETSQRFSSVCQAKLSKTNSQGRITKLAVRQKRSDSD 1750
IRPQRAAQRPWAKCKQGESKVTRNVKPTTQSHVPLPAGTDVIPAHQFTDE 1800
LAAYEPTPPLSPELPGSCKKEPKKKRPSFPKARRRSVKLGHVALDSKSHN 1850
QHDSVQIIASVSDLKSMDEPLLKEVNDLDKEDSKKDTLVDVVFKKALKEF 1900
RQHIFSPTESALANDDGKSIRTKDLYALFEQILERTKLEQRDSLGKSPV 1950
RVWVNTFKVFLDETHKEFRTSDCTATKVPKTERKKRRKETDLVEEHSHGH 2000
IFKATQYSIFTYCEYCSELIWIMDRASVCKLCKYACHKKCCLKTTAKCSK 2050
KYDPELSSRQPGVRLSRLPKEDRTVPLVVERLINYIEHHGLYTEGIYRKS 2100
GSTNKIKELRQGLDTDARSVWLDDYWIHRVIASVFKQWLRDLPHPLNTTEL 2150
YEESLRANGILQERKESTIEGVTSVIDQLSRTHLWTLSRALIFHLVRIALQED 2200
TRDRSARALAIVFAPCILRCPDTTOPLGSVQDISRTTTCVRLIVVSQMNK 2250
YIARLEDISSLEFAENKANTRLSLILRLSNCKGRIRRGSYPCPSSPVVVRL 2300
PSVSDVSENTLTRRAAMLSTDITSQQQARMQQEENVLTQPQIRELQKSKCSL 2350
TTEMLVLEPRASDDSTLESEASIGTADSSEMLDNSSETAISESERSSLAL 2400
SSLKTAGKSEPSSKLERQLKMQQDSLDVVDSSVSSLCLSWTASSHGTRKL 2450
FQIYSKSPYTRAASGHEALGMEGPLGQTKFLEDKPQFISRGTFRPERGKQ 2500
KLRSVKHSPQKTKSTPEGTVMSGRAKTVDPDCTSWQQLALFGMMEPHV 2548
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MYOSIN IXA AND CYCLIC NUCLEOTIDE GATED CHANNEL-15 (CNGC-15) POLYNUCLEOTIDES, POLYPEPTIDES, COMPOSITIONS, METHODS, AND USES THEREOF

FIELD OF THE INVENTION

This invention relates to the fields of molecular biology and pharmaceutical research. More specifically, this invention relates to the identification and recombinant expression of two new genes, cyclic nucleotide gated channel-15 (CNGC-15) and Myosin IXa. Accordingly, isolation of the human myosin IXa gene will prove useful in the study, diagnosis and treatment of Bardet-Biedl Syndrome, Usher Syndrome and related conditions.

BACKGROUND OF THE INVENTION

Usher Sydrome typel (USH1) is characterized by a profound congenital sensoneural hearing loss, vestibular dysfunction and prepubescent onset retinitis pigmentosa. Family studies indicated that three genes with different chromosomal locations are responsible for USH1; a defect in any one of these genes causes the disease. Of these, the gene USH1B mapped to a region homologous to the murine region containing the mouse deafness mutant *shaker-1* which results from mutations in myosin VIIa. Subsequent work confirmed that myosin VIIa is the cause of Usher Sydrome type 1B and localized the myosin VIIa protein to the receptor cells of the inner ear (Hasson *et al.* (1995) *Genomics 36*:431-439) and the connecting cilia of photoreceptor cells in the retina (Liu *et al.* (1997) *Cell Motil. Cytoskel. 37*:240-252).

Retinitis pigmentosa is also associated with Bardet-Biedl Syndrome. Bardet-Biedl Syndrome is further characterized by obesity, retinal degeneration, syndactyly and/or polydactyly, hypogenitalism and mental retardation (Schachat *et al.* (1982) *Arch. Ophthalmol. 100*:285-288; Green *et al.* (1989) *N. Engl. J. Med. 321*:1002-1009) and can result from aberrations in one of at least four different genes. Other publications relating to myosin IXa include Bruford *et al.* (1997) *Genomics 41*:93-99 and Bement *et al.* (1994) *Proc. Natl. Acad. Sc. USA 91*:6549-6553.

All references cited herein are incorporated by reference.

SUMMARY OF THE INVENTION

The present invention discloses the amino acid and nucleic acid sequences of a new Cyclic nucleotide gated channel (CNGC) and Myosin that map to the region of the human chromosome associated with Bardet-Biedl Syndrome. CNGCs comprise a family of multimeric protein ion channels that open in response to the binding of a cyclic nucleotide to an intracellular domain. The two new proteins, CNGC-15 and Myosin IXa, are useful in the study, diagnosis and treatment of Bardet-Biedl Syndrome and Usher Syndrome. Other indications that can be treated by CNGC-15 and/or Myosin IXa polypeptides, or agonists or antagonists include hearing loss, retinis pigmentosa, obesity, hypogonadism, sterility, polydactyly, brachydactyly, syndactyly, mental retardation, renal abnormalities, hypertension, diabetes and cardiovascular abnormalities.

Compositions and methods for expressing cyclic nucleotide gated channel-15 (CNGC-15) and Myosin IXa are provided. The compositions comprise CNGC-15 and Myosin IXa polypeptides and derivatives thereof, nucleotide sequences, expression cassettes, transformed cells and antibodies to these polypeptides. Methods for the expression and detection of CNGC-15 and Myosin IXa nucleotides and polypeptides and compositions for the treatment of these conditions are provided.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 sets forth the amino acid sequence of the human Myosin IXa polypeptide (SEQ ID NO: 1).

Figure 2 sets forth the nucleotide sequence of the human Myosin IXa cDNA (SEQ ID NO: 2). The ATG translational intiation codon at position 243 and the TGA translational stop codon at position 7887 are underlined.

Figure 3 sets forth the nucleotide sequence of a human CNGC-15 partial cDNA (SEQ ID NO: 3).

DETAILED DESCRIPTION OF THE INVENTION

The present invention is drawn to compositions and methods useful in the treatment of Bardet-Biedl Syndrome, Usher Syndrome, hearing loss, retinis pigmentosa, obesity, hypogonadism, sterility, polydactyly, brachydactyly, syndactyly, mental retardation, renal abnormalities, hypertension, diabetes and cardiovascular abnormalities. More particularly, two new polypeptides and the genes encoding them, CNGC-15 and Myosin IXa, have been identified that are useful in the treatment of these and a variety of other conditions. The human Myosin IXa polypeptide and cDNA are shown in Figures 1 and 2, as SEQ ID NO: 1 and SEQ ID NO: 2, respectively. The human CNGC-15 partial cDNA is shown in Figure 3 as SEQ ID NO: 3.

Thus, it is an object of the invention to provide native CNGC-15 and Myosin IXa substantially free of other human proteins.

Another object of the invention is to provide polypeptides that are mutants, fragments and derivatives of CNGC-15 or Myosin Ixa. Regions of particular importance of Myosin IXa include: the open reading frame (nucleotides 243-7886 of SEQ ID NO: 2) encoding a 2548 amino acid protein (SEQ ID NO: 1); the N-terminal extension (residues 1-146 of SEQ ID NO: 1) encoded by nucleotides 243-680 of SEQ ID NO: 2; the head domain (residues 147-719 of SEQ ID NO: 1) encoded by nucleotides 683-2399 of SEQ ID NO: 2; the ATP binding loop (residues 239-246 of SEQ ID NO: 1) encoded by nucleotides 959-977 of SEQ ID NO: 2; the minimal length 2nd insert (residues 720-835 of SEQ ID NO: 1) encoded by nucleotides 2402-2747 of SEQ ID

NO: 2; the light chain binding domain (residues 972-1166 of SEQ ID NO: 1) encoded by nucleotides 3158-3740 of SEQ ID NO: 2; and the GAP domain (residues 2074-2219) of SEQ ID NO: 1 encoded by nucleotides 6473-6899 of SEQ ID NO: 2.

Accordingly, the invention provides a Myosin IXa polypeptide comprising an amino acid sequence selected from the group consisting of:

a) SEQ ID NO: 1;

b) residues 1-146 of SEQ ID NO: 1;

c) residues 147-719 of SEQ ID NO: 1;

d) residues 239-246 of SEQ ID NO: 1;

e) residues 720-835 of SEQ ID NO: 1;

f) residues 972-1166 of SEQ ID NO: 1;

g) residues 2074-2219 of SEQ ID NO: 1;

h) a polypeptide having at least about 61% homology to SEQ ID NO:1;

i) a polypeptide having at least about 95% homology to an amino acid sequence of b), c), d), e), f) or g); and

j) a polypeptide of at least about 90 contiguous residues of SEQ ID NO: 1.

The Myosin IXa polypeptides of the invention will have at least about 61% homology to SEQ ID NO:1, preferably at least about 70% homology, more preferably at least about 80% homology and most preferably at least about 90% homology. The polypeptides of b), c), d), e), f) or above, will have at least about 95% homology to SEQ ID NO:1, more preferably at least about 96% homology, even more preferably at least about 97% homology and most preferably at least about 98% homology.

Similarly, the invention provides a CNGC-15 polypeptide comprising an amino acid sequence selected from the group consisting of :

a) a polypeptide encoded by SEQ ID NO: 3;

b) a polypeptide encoded by nucleotides 369-1307 of SEQ ID NO: 3;

c) a polypeptide having at least about 80% homology to the polypeptide encoded by nucleotides 369-1307 of SEQ ID NO: 3; and

d) a polypeptide of at least about 20 contiguous residues of the polypeptide encoded by nucleotides 369-1307 of SEQ ID NO: 3.

The CNGC-15 polypeptides of the invention will have at least about 70% homology to a), b), c) or d) above, preferably at least about 80% homology, more preferably at least about 85% homology and most preferably at least about 90% homology.

In another aspect of the invention provides a chimeric polypeptide comprising a CNGC-15 polypeptide, or fragment thereof and a polypeptide of interest. Similarly, the invention provides a chimeric polypeptide comprising a Myosin IXa polypeptide, or fragment thereof, fused to a polypeptide of interest. Nucleotide sequences encoding chimeric CNGC-15 and Myosin IXa polypeptides are also provided.

Yet another object of the invention is to provide polynucleotides that encode the mutants, fragments, and derivatives, as well as the native CNGC-15 and Myosin IXa. Accordingly, the invention provides an isolated nucleotide sequence selected from the group consisting of:

a) a nucleotide sequence encoding a native Myosin IXa polypeptide;

b) a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 1;

c) the nucleotide sequence of SEQ ID NO: 2;

d) a nucleotide sequence comprising at least about 390 contiguous bases of SEQ ID NO: 2;

e) a nucleotide sequence comprising nucleotides 243-7085 of SEQ ID NO: 2;

f) a nucleotide sequence comprising nucleotides 243-680 of SEQ ID NO: 2;

g) a nucleotide sequence comprising nucleotides 683-2399 of SEQ ID NO: 2;

h) a nucleotide sequence comprising nucleotides 959-977 of SEQ ID NO: 2;

i) a nucleotide sequence comprising nucleotides 2402-2747 of SEQ ID NO: 2;

j) a nucleotide sequence comprising nucleotides 3158-3740 of SEQ ID NO: 2; and

k) a nucleotide sequence comprising nucleotides 6473-6899 of SEQ ID NO: 2.

The invention similarly provides an isolated nucleotide sequence selected from the group consisting of:

a) a nucleotide sequence encoding a native CNGC-15 polypeptide;

b) a nucleotide sequence encoding the polypeptide encoded by nucleotides 369-1307 of SEQ ID NO: 3;

c) the nucleotide sequence of SEQ ID NO: 3;

d) a nucleotide sequence comprising at least 45 contiguous nucleotides of the sequence of SEQ ID NO:3; and

e) a nucleotide sequence that hybridizes under stringent conditions to a denatured DNA having a nucleotide sequence of a), b), c) or d).

These polynucleotides can be operably linked to heterologous promoters to form expression cassettes. The expression cassettes can be introduced into suitable host cells for expression of CNGC-15 and/or Myosin IXa polypeptides and derivatives thereof.

Another object of the invention is to provide a transformed cell transiently expressing or having stably incorporated into its genome an expression vector comprising a promoter operably linked to a nucleotide sequence encoding a CNGC-15 or Myosin IXa polypeptide, or a fragment, derivative mutant or fusion thereof.

A further object of the invention is to provide a method for producing a CNGC-15 or Myosin IXa polypeptide or a derivative thereof, comprising:

a) providing a host cell transiently or stably transformed with an expression vector comprising a promoter operably linked to a nucleotide sequence encoding said CNGC-15 or Myosin IXa polypeptide or derivative thereof; and

b) culturing the host cell under conditions which allow expression of said polypeptide.

Another object of the invention is to provide a method for detecting CNGC-15 polynucleotides. The method comprises:

a) providing a nucleic acid probe which hybridizes to a nucleotide encoding a CNGC-15 polypeptide or a mutant, fragment or derivative thereof;

b) contacting the probe with a sample of polynucleotides under hybridizing conditions to form a duplex; and

c) detecting said duplexes.

A further object of the invention is to provide a method for detecting unconventional Myosin polynucleotides. The method comprises:

a) providing a nucleic acid probe which hybridizes to a nucleotide encoding a Myosin IXa or a mutant, fragment or derivative thereof;

b) contacting the probe with a sample of polynucleotides under hybridizing conditions to form a duplex; and

c) detecting said duplexes.

Yet another object of the invention is to provide antibodies to the CNGC-15 and Myosin IXa polypeptides.

Another object of the invention is to provide a method for detecting CNGC-15 polypeptides. The method comprises:

a) providing an antibody that binds to a CNGC-15 polypeptide;

b) contacting the antibody to a sample under binding conditions to form a duplex; and

c) detecting said duplexes.

Still another object of the invention is to provide a method for detecting Myosin IXa polypeptides. The method comprises:

a) providing an antibody that binds to a Myosin IXa polypeptide;

b) contacting the antibody to a sample under binding conditions to form a duplex; and

c) detecting said duplexes.

The invention further provides a method for treating Bardet-Biedl Syndrome, Usher Syndrome, hearing loss, retinis pigmentosa, obesity, hypogonadism, sterility, polydactyly, brachydactyly, syndactyly, mental retardation, renal abnormalities, hypertension, diabetes and cardiovascular abnormalities, comprising administering a therapeutically effective amount of a CNGC-15 or Myosin IXa polypeptide, or derivative thereof to a subject in need of such treatment. In still another aspect, the invention provides a composition comprising CNGC-15 or Myosin IXa or an active derivative thereof, and a pharmaceutically acceptable carrier.

Modes for Carrying Out the Invention

A. Definitions

As used herein, the terms "native CNGC-15" and "native Myosin IXa" refer to the polypeptides found in nature. for example, native CNGC-15 and native Myosin IXa are polypeptides with substantial amino acid sequence identity to SEQ ID NO: 1 and SEQ ID NO: 3, respectively. This definition includes allelic variants and other naturally occurring modifications of SEQ ID NO: 1 and SEQ ID NO: 3.

"CNGC-15 polypeptides" and "Myosin IXa polypeptides" include mutants, fragments, derivatives and fusions as well as the native CNGC-15 or Myosin IXa.

"Mutants" of the native CNGC-15 or Myosin IXa are polypeptides having an amino acid sequence which retain at least about 50% amino acid sequence identity with SEQ ID NO: 1 or SEQ ID NO: 3, respectively; more typically, at least about 60%; even more typically, at least about 80%. Preferably mutants will retain at least about 85% amino acid sequence identity with SEQ ID NO: 1 or SEQ ID NO: 3; more preferably, at least about 90%; even more preferably, at least about 95%. These differences may be conservative amino acid substitutions, insertions or deletions in the amino acid sequence.

"Fragments" possess the same amino acid sequence of the native or mutant CNGC-15 or Myosin IXa polypeptides except the fragments lack the amino and/or carboxyl terminal sequences of the native or mutant polypeptide.

"Derivatives" possess the same amino acid sequence of the native, mutant or fragment CNGC-15 and Myosin IXa, but may contain amino acid substitutes, glycosylated residues or other chemical modifications.

"Fusions" or "chimeric polypeptides" are mutants, fragments, or the native CNGC-15 or Myosin IXa that also include amino and/or carboxyl terminal amino acid extensions. For example, Myosin Ixa fragments may be fused with fragments of other unconventional myosins. Unconventional myosins are members of the myosin superfamily that display the general head, neck and tail domain structure of conventional myosins (Mooseker *et al.* (1995) *Annu. Rev. Cell. Dev. Biol. 11*:633-75), but differ from conventional myosins by changes in these and/or the inclusion of additional functional domains. The head region shared among all myosin molecules consists of conserved amino acid sequences that span approximately 80 kDa while the neck region contains conserved motifs that bind one to six light chains of protein from the calmodulin/EF-hand family (Bahler *et al.* (1996) *Curr. Op. Cell. Biol. 8*:18-22). It is the unconventional myosin tail domains that show the most variation from conventional myosin and from each other. For example, although myosin Va has a coiled coil tail domain, as does conventional myosin, it also contains a PEST site (required for calpain cleavage) and a globular C-terminal domain which may be used for interactions with cargo or docking proteins. Myosin VIIa also has a coiled coil tail domain, however it contains additional domains different from those found in myosin Va, such as the talin domain which is thought to be active in binding plasma membranes. As a third example, the class IX myosins (composed of rat myr5 and human myosin IXb) have even less similarity to conventional myosin and to the other nine classes of unconventional myosins. These molecules lack a coiled coil domain in the tail region, but do contain a GAP (GTP activating protein) domain which increases the GTPase activity of rhoA and Cdc42 (Reinhard *et al.* (1995) *EMBO J. 14*:697-704). Class IX myosins also have two large insertions in the conserved head domain that are absent in conventional and other unconventional myosins, and may act as regulatory domains affecting the mechanochemical properties of the protein. Despite the variation among these three examples of unconventional myosin

classes, all classes bind actin and have either been shown to be or are postulated to act as molecular motors (Wang *et al.* (1996) *Science 273*:660-663), thereby interacting with the actin cytoskeleton. The characterization and chromosomal mapping of myosin Va and myosin VIIa suggested that these molecules may be responsible for specific human diseases. Indeed, defects in myosinVa are associated with Griscelli disease (Pastural *et al.* (1997) *Nature Genet. 16*:289-292), while mutations in myosin VIIa are responsible for Usher Sydrome type1B (Weil *et al.* (1995) *Nature 374*:60-61.

The number or type of the amino acid substitutions is not critical, nor is the length or number of the amino acid deletions, or amino acid extensions that are incorporated in the CNGC-15 or Myosin IXa polypeptides. However, all of these polypeptides will exhibit at least about 20% of one of the activities of the native CNGC-15 or Myosin IXa. More typically, the polypeptides exhibit at least about 40%, even more typically the polypeptides exhibit at least about 60% of one of the native CNGC-15 or Myosin IXa activities. All these polypeptides will retain at least about 50% amino acid identity with SEQ ID NO: 1 or SEQ ID NO: 3; more typically, at least about 60%; even more typically, at least about 80%. Preferably, these polypeptides will retain at least about 85% amino acid sequence identity with SEQ ID NO: 1 or the amino acid sequence encoded by SEQ ID NO: 3; more preferably, at least about 90%; even more preferably, at least about 95%.

Myosin IXa activities include immunological activities, ATP binding, ATPase activity (Shyamata *et al.* (1990) J Cell Biol 110:1137-1147), zinc binding (Reinhard *et al.* (1995) EMBO J 14:697-704; Quest et . (1994) J Biol Chem 269:2961-2970) calmodulin/EF binding (Glenney *et al.* (1980) J Biol Chem 255:10551-10554; Cheney, (1992) Current Opin Cell Biol 4:27-35), binding to G-coupled receptors, GTPase activity (Reinhard, *Supra*; Ridley (1992) Cell 70:401-410), membrane binding (Reizes (1994) PNAS USA 91:6439-6443) phorbolester binding (Reinhard, *Supra*), modulation of cell to cell interactions, calcium release and cytoskeletal rearrangements, actin binding (Ruppert *et al.* (1993) J Cell Biol 120:1393-1403; Ridley *et al.* (1992) Cell 70:389-399; Ridley *et al.* (1992) Cell 70:401-410), formation of focal complexes (Ridley, *Supra*), synthesis of phosphatidylinositol-4,5-bis-

phosphate, activation of transcription and DNA synthesis and transduction of chemical energy into mechanical force along actin filaments (Collins *et al.* (1990) J Cell Biol 110:1137-1147).

"Hybridization" refers to the association of two nucleic acid sequences to one another by hydrogen bonding. Factors that affect this bonding are discussed in Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual, Second Edition*, Volume 2, chapter 9, pages 9.47 to 9.57. A nucleic acid probe is said to "hybridize" with SEQ ID NO: 2 or SEQ ID NO: 3 if the probe can form a duplex or double stranded complex, which is stable enough to be detected. Hybridization of the probe to a polynucleotide of SEQ ID NO: 2 or SEQ ID NO: 3 depends on (1) the sequence of the nucleic acid probe and (2) the hybridization conditions. The sequence of the probe need not be exactly complementary. For example, a non-complementary nucleotide sequence may be attached to the 5′ end of the probe, with the remainder of the probe sequence being complementary to SEQ ID NO: 2 or SEQ ID NO: 3. Alternatively, non-complementary bases or longer sequences can be interspersed into the probe, provided that the probe sequence has sufficient complementarity with SEQ ID NO: 2 or SEQ ID NO: 3 to hybridize therewith and thereby form a duplex which can be detected. The exact length and sequence of the probe will depend on the hybridization conditions, such as temperature, salt condition and the like. For example, for diagnostic applications, depending on the complexity of the analyte sequence, the nucleic acid probe typically contains 15-25 or more nucleotides, although it may contain fewer nucleotides. Short primers generally require cooler temperatures to form sufficiently stable hybrid complexes with the template.

Two sequences can be aligned using the methods and computer programs described above, and include BLAST, available over the world wide web at http://ww.ncbi.nlm.nih.gov/BLAST/. Another alignment algorithm is Fasta, available in the Genetics Computing Group (GCG) package, Madison, Wisconsin, USA, a wholly owned subsidiary of Oxford Molecular Group, Inc. Other techniques for alignment are described in Doolittle, Methods in Enzymology, *Supra*. Preferably, an alignment program that permits gaps in the sequence is utilized to align the sequences. The Smith-Waterman is one

type of algorithm that permits gaps in sequence alignments. See Meth. Mol. Biol. 70: 173-187 (1997). Also, the GAP program using the Needleman and Wunsch alignment method can be utilized to align sequences. An alternative search strategy uses MPSRCH software, which runs on a MASPAR computer. MPSRCH uses a Smith-Waterman algorithm to score sequences on a massively parallel computer. This approach improves ability to pick up distantly related matches, and is especially tolerant of small gaps and nucleotide sequence errors. EST-encoded amino acid sequences can be used to search both protein and DNA databases.

Of interest is the BestFit program using the local homology algorithm of Smith Waterman (Advances in Applied Mathematics 2: 482-289 (1981)) to determine sequence identity. The gap generation penalty will generally range from about 1 to about 5, usually, about 2 to about 4 and in many embodiments will be about 3. The gap extension penalty will generally range from about 0.01 to 0.20 and in many instances will be 0.10. The program has default parameters determined by the sequences inputted to be compared. Preferably, the sequence identity is determine used the parameters determined by the program. This program is available from Genetics Computing Group (GCG), Madison, Wisconsin, USA.

Additional factors used to determine sequence identity are: percentage of the alignment region length where the strongest alignment is found, percent sequence identity, and p value.

The percentage of the alignment region length is calculated by counting the number of residues of the individual sequence found in the region of strongest alignment. This number is divided by the total residue length of the query sequence to find a percentage. An example is shown below:

```
Sequence A:  ASNPERTMIPVTRVGLIRYM
               |  ||| |||| |||
Sequence B:  YMMTEYLAIPV.RVGLPRYM
             1  5  10  15
```

The region of alignment begins at amino acid 9 and ends at amino acid 19. The total length of the query sequence is 20 amino acids. The percent of the alignment region length is 11 divided by 20 or 55%.

Percent sequence identity is calculated by counting the number of amino acid matches between the two sequences and dividing total number of matches by the number of residues of the individual sequence found in the region of strongest alignment. For the example above, the percent identity would be 10 matches divided by 11 amino acids, or approximately, 90.9%

For the alignment results to be considered high similarity, the percent of the alignment region length, typically, is at least about 55% of total length one of the sequences; more typically, at least about 58%; even more typically; at least about 60% of the total residue length of one of the sequences. Usually, percent length of the alignment region can be as much as about 62%; more usually, as much as about 64%; even more usually, as much as about 66%. Typically, the percent length of alignment region is at least about 75% of the total length of one of the sequences; more typically, at least about 80%; even more typically, about 85%, about 90%, about 95%, about 98%, or 99%.

P value is the probability that the alignment was produced by chance. For a single alignment, the p value can be calculated according to Karlin *et al.*, Proc. Natl. Acad. Sci. 87: 2264 (1990) and Karlin *et al.*, Proc. Natl. Acad. Sci. 90: (1993). The p value of multiple alignments using the same query sequence can be calculated using an heuristic approach described in Altschul *et al.*, Nat. Genet. 6: 119 (1994). Alignment programs such as BLAST program can calculate the p value.

The two sequences, typically, exhibit a p value is less than or equal to about 10^{-2}; more usually; less than or equal to about 10^{-3}; even more usually; less than or equal to about 10^{-4}. More typically, the p value is no more than about 10^{-5}; more typically; no more than or equal to about 10^{-10}; even more typically; no more than or equal to about 10^{-15}. Preferably, the p value is no more than about 10^{-20}; more preferably, no more than about 10^{-30}; even more preferably, no more than about 10^{-40}, about 10^{-50}, or 10^{-60}.

The boundaries of the region where the sequences align can be determined according to Doolittle, Methods in Enzymology, *Supra*; BLAST or

FAST programs; or by determining the area where the sequence identity is highest.

Another factor to consider for determining identity or similarity is the location of the similarity or identity. Strong local alignment can indicate similarity even if the length of alignment is short. Sequence identity scattered throughout the length of the one sequence also can indicate a similarity between two sequences.

"Stringency" refers to conditions in a hybridization reaction that favor association of very similar sequences over sequences that differ. Factors affecting the stringency of hybridization are well known to those skilled in the art and are discussed in Sambrook *et al.* above at page 9.50.

In general, convenient hybridization temperatures in the presence of 50% formamide are 42°C for a probe with is 95% to 100% homologous to the target fragment, 37°C for 90% to 95% homology, and 32°C for 85% to 90% homology. For lower homologies, formamide content should be lowered and temperature adjusted accordingly, using the equation above. If the homology between the probe and the target fragment are not known, the simplest approach is to start with both hybridization and wash conditions which are nonstringent. If nonspecific bands or high background are observed after autoradiography, the filter can be washed at high stringency and reexposed. If the time required for exposure makes this approach impractical, several hybridization and/or washing stringencies should be tested in parallel.

Typically, stringent conditions will be those in which the salt concentration is less than about 1.5 M Na ion, typically about 0.01 to 1.0 M Na ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (*e.g.*, 10 to 50 nucleotides) and at least about 60°C for long probes (*e.g.*, greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. Exemplary low stringency conditions include hybridization with a buffer solution of 30 to 35% formamide, 1 M NaCl, 1% SDS (sodium dodecyl sulphate) at 37°C, and a wash in 1X to 2X SSC (20X SSC = 3.0 M NaCl/0.3 M trisodium citrate) at 50 to 55°C. Exemplary moderate stringency conditions include hybridization in 40 to 45% formamide, 1 M NaCl, 1% SDS at 37°C,

and a wash in 0.5X to 1X SSC at 55 to 60°C. Exemplary high stringency conditions include hybridization in 50% formamide, 1 M NaCl, 1% SDS at 37°C, and a wash in 0.1X SSC at 60 to 65°C.

A composition containing A is "substantially free of" B when at least about 85% by weight of the total A+B in the composition is A. Preferably, A comprises at least about 90% by weight of the total of A+B in the composition, more preferably at least about 95% or even 99% by weight.

The term "heterologous" refers to two biological components that are not found together in nature. The components may be host cells, genes, or regulatory regions, such as promoters. Although the heterologous components are not found together in nature, they can function together, as when a promoter heterologous to a gene is operably linked to the gene. Heterologous components may be from the same or different organisms. Another example is where a human CNGC-15 or Myosin IXa coding sequence is heterologous to a mouse host cell.

A "promoter" is a DNA sequence that initiates and regulates the transcription of a coding sequence when the promoter is operably linked to the coding sequence. A promoter is "heterologous" to the coding sequence when the promoter is not operably linked to the coding sequence in nature. For example, a human CNGC-15 Myosin IXa promoter would comprise an expression cassette wherein a nucleotide sequence encoding CNGC-15 is operably linked to a heterologous promoter. A "native" promoter is operably linked to the coding sequence in nature.

An "origin of replication" is a polynucleotide sequence that initiates and regulates replication of polynucleotides, such as an expression vector. The origin of replication behaves as an autonomous unit of polynucleotide replication within a cell, capable of replication under its own control. With certain origins of replication, an expression vector can be reproduced at a high copy number in the presence of the appropriate proteins within the cell. Examples of origins are the 2μ and autonomously replicating sequences, which are effective in yeast; and the viral T-antigen, effective in COS-7 cells.

Host cells capable of producing CNGC-15 and/or Myosin IXa polypeptides are cultured "under conditions allowing expression." Such conditions allow transcription and translation of the DNA molecule encoding the CNGC-15 and/or Myosin IXa polypeptide. These conditions include cultivation temperature, oxygen concentration, media composition, pH, *etc.* For example, if the trp promoter is utilized in the expression vector, the media will lack tryptophan to trigger the promoter and induce expression. The exact conditions will vary from host cell to host cell and from expression vector to expression vector.

As used herein, the term "antibody" refers to a polypeptide or group of polypeptides composed of at least one antibody combining site. An "antibody combining site" is the three-dimensional binding space with an internal surface shape and charge distribution complementary to the features of an epitope of an antigen, which allows a binding of the antibody with the antigen. "Antibody" includes, for example, vertebrate antibodies, hybrid antibodies, chimeric antibodies, altered antibodies, univalent antibodies, the Fab proteins, and single domain antibodies.

"Myosin IXa-modulated disorders" and "CNGC-15 modulated-disorders" include, but are not limited to Bardet-Biedl Syndrome, Usher Syndrome, hearing loss, retinis pigmentosa, obesity, hypogonadism, sterility, polydactyly, brachydactyly, syndactyly, mental retardation, renal abnormalities, hypertension, diabetes and cardiovascular abnormalities

B. General Method

This invention provides the amino acid and nucleotide sequences of CNGC-15 and Myosin IXa. With these disclosed sequences, nucleic acid probe assays and expression cassettes and vectors for CNGC-15 and Myosin IXa polypeptides can be produced. The expression vectors can be transformed into host cells to produce CNGC-15 and/or Myosin IXa polypeptides. The purified polypeptides can be used to produce antibodies to detect CNGC-15 or Myosin IXa. Also, the host cells or extracts can be utilized for biological assays to isolate agonists or antagonists.

Nucleic Acid CNGC-15 Probe Assays

mRNA levels in different cell types can be detected with nucleic acid probe assays. For example, PCR, branched DNA probe assays, or blotting techniques utilizing nucleic acid probes substantially identical or complementary to SEQ ID NO: 2 or SEQ ID NO: 3 can determine the presence of CNGC-15 or Myosin IXa cDNA or mRNA.

For genomic analysis or detection of denatured DNA, the nucleic acid probes will hybridize to a nucleotide sequence encoding the amino acid sequence shown in SEQ ID NO: 1 or encoded by SEQ ID NO: 3, or the complement of a sequence encoding SEQ ID NO: 1 or the polypeptide encoded by SEQ ID NO: 3. Though many different nucleotide sequences will encode the amino acid sequences, SEQ ID NO: 2 and SEQ ID NO: 3 are preferred because they are the actual sequences present in human cells. For single-stranded cDNA detection, the nucleic acid probe will hybridize to the complement of a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 1 or encoding the polypeptide encoded by nucleotides 369-1307 of SEQ ID NO: 3 or to a complement of SEQ ID NO: 2 or SEQ ID NO: 3. For mRNA detection, the nucleic acid probe will hybridize to SEQ ID NO: 2 or SEQ ID NO: 3 or to a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 1 or encoding the polypeptide encoded by SEQ ID NO: 3. The nucleic acid probe sequences need not be identical to SEQ ID NO: 2, SEQ ID NO: 3 or their complement.

Probes are typically at least about 20 nucleotides, more preferably at least about 30 nucleotides. The probes may be produced by synthetic procedures, such as the triester method of Matteucci *et al.* (1981) *J. Am. Chem. Soc. 103*:3185, or according to Urdea *et al.* (1983) *Proc. Natl. Acad. Sci. USA 80*:7461, or using commercially available automated oligonucleotide synthesizers. One example of a nucleotide hybridization assay is described in Urdea *et al.* PCT WO92/02526 and Urdea *et al.* U.S. Patent NO. 5,124,246, herein incorporated by reference. Other methods of hybridization and detection are known to those skilled in the art.

Alternatively, the Polymerase Chain Reaction (PCR) is another well-known means for detecting small amounts of target nucleic acids. The assay is described in Mullis *et al.* (1987) *Meth. Enzymol. 155*:335-350; U.S. Patent No. 4,683,195; and U.S. Patent No. 4,683,202, incorporated herein by reference. Also, mRNA, cDNA and genomic DNA can be detected by traditional blotting techniques described in Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual* (New York, Cold Spring Harbor Laboratory).

Expression of CNGC-15 and Myosin IXa Polypeptides

Preferably, CNGC-15 and Myosin IXa polypeptides are produced by recombinantly engineered host cells. These host cells are constructed by the introduction of a expression vector comprising a promoter operably linked to a CNGC-15 or Myosin IXa polypeptide coding sequence.

Such coding sequences can be constructed by synthesizing the entire gene or by altering existing CNGC-15 or Myosin IXa polypeptide coding sequences. CNGC-15 and Myosin IXa polypeptides can be divided into four general categories: mutants, fragments, fusions, and the native CNGC-15 or Myosin IXa polypeptides. The CNGC-15 polypeptides are those that occur in nature. The amino acid sequence of such polypeptides may vary slightly from SEQ ID NO: 1. The native CNGC-15 and Myosin IXa polypeptide coding sequence can be selected based on the amino acid sequence shown in SEQ ID NO: 1 or the amino acid sequence predicted by the open reading frame of SEQ ID NO: 3. For example, synthetic genes can be made using codons preferred by the host cell to encode the desired polypeptide. (See Urdea *et al.* (1983) *Proc. Natl. Acad. Sci. USA 80*:7461). Alternatively, the desired native CNGC-15 and Myosin IXa polypeptide coding sequences can be cloned from nucleic acid libraries. Techniques for producing and probing nucleic acid sequence libraries are described, for example, in Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual* (New York, Cold Spring Harbor Laboratory). Other recombinant techniques, such as site specific mutagenesis, PCR, enzymatic digestion and ligation, can also be used to construct the desired CNGC-15 polypeptide coding sequence.

The native CNGC-15 and Myosin IXa polypeptide coding sequences can be modified to create the other classes of CNGC-15 and Myosin IXa polypeptides. For example, mutants can be created by making conservative amino acid substitutions that maintain or enhance native CNGC-15 or Myosin IXa activity. The following are examples of conservative substitutions: Gly ⇔ Ala; Val ⇔ Ile ⇔Leu; Asp ⇔ Glu; Lys ⇔ Arg; Asn ⇔ Gln; and Phe ⇔Trp ⇔ Tyr. Mutants can also contain amino acid deletions or insertions compared to the native CNGC-15 or Myosin IXa polypeptides. Mutants may include substitutions, insertions, and deletions of the native polypeptides.

Mutants will retain at least about 20% of the one of the activities of the native CNGC-15 or Myosin IXa. The coding sequence of mutants can be constructed by *in vitro* mutagenesis of the native coding sequences.

A subset of mutants is a group of polypeptides with the non-disulfide bond participating cysteines substituted with a neutral amino acid, generally, with serines. These mutants may be stable over a broader temperature range than native CNGC-15 or Myosin IXa polypeptides. See, for example, Mark *et al.*, U.S. Patent No. 4,959,314.

Fragments differ from mutant or native CNGC-15 or Myosin IXa polypeptides by amino and/or carboxyl terminal amino acid deletions. The number of amino acids that are truncated is not critical as long as the CNGC-15 or Myosin IXa fragment retains at least about 20% of the one of the activities of the native CNGC-15 or Myosin IXa polypeptide. The coding sequence of such fragments can be easily constructed by cleaving the unwanted nucleotides from the mutant or native CNGC-15 or Myosin IXa polypeptide coding sequences.

Fusions are fragment, mutant, or native CNGC-15 or Myosin IXa polypeptides with additional amino acids at either or both of the termini. The additional amino acid sequence is not necessarily homologous to sequence found in native polypeptides. The fusions, just as all CNGC-15 and Myosin IXa polypeptides, retain at least about 20% of one of the activities of the native CNGC-15 or Myosin IXa polypeptides. Coding sequence of the fusions can be constructed by ligating synthetic polynucleotides encoding the additional amino acids to fragment, mutant, or native coding sequences.

Activities of the CNGC-15 and Myosin IXa polypeptides can be determined by the methods described *infra*.

Expression Vectors

At the minimum, an expression vector will contain a promoter which is operable in the host cell and operably linked to a CNGC-15 or Myosin IXa coding sequence. Sequences that modulate gene expression, such as enhancers and binding sites for inducers or repressors may be present. Expression vectors may also include signal sequences, terminators, selectable markers, origins of replication, and sequences homologous to host cell sequences. These additional elements are optional but can be included to optimize expression.

Functional non-natural promoters may also be used, for example, synthetic promoters based on a consensus sequence of different promoters. Also, effective promoters can contain a regulatory region linked with a heterologous expression initiation region. Examples of hybrid promoters are the *E. coli* lac operator linked to the *E. coli* tac transcription activation region; the yeast alcohol dehydrogenase (ADH) regulatory sequence linked to the yeast glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) transcription activation region (U.S. Patent Nos. 4,876,197 and 4,880,734, incorporated herein by reference); and the cytomegalovirus (CMV) enhancer linked to the SV40 (simian virus) promoter.

A CNGC-15 or Myosin IXa polypeptide coding sequence may also be linked in reading frame to a signal sequence. The signal sequence fragment typically encodes a peptide comprised of hydrophobic amino acids which directs the CNGC-15 or Myosin IXa polypeptide to the cell membrane or other subcellular compartment. Preferably, there are processing sites encoded between the leader fragment and the gene or fragment thereof that can be cleaved either *in vivo* or *in vitro*. DNA encoding suitable signal sequences can be derived from genes for secreted endogenous host cell proteins, such as the yeast invertase gene (EP 12 873; JP 62,096,086), the A-factor gene (U.S. Patent No. 4,588,684), interferon signal sequence (EP 60 057).

A preferred class of secretion leaders, for yeast expression, are those that employ a fragment of the yeast alpha-factor gene, which contains both a "pre" signal sequence, and a "pro" region. The types of alpha-factor fragments that can be employed include the full-length pre-pro alpha factor leader (about 83 amino acid residues) as well as truncated alpha-factor leaders (typically about 25 to about 50 amino acid residues) (U.S. Patent Nos. 4,546,083 and 4,870,008, incorporated herein by reference; EP 324 274). Additional leaders employing an alpha-factor leader fragment that provides for secretion include hybrid alpha-factor leaders made with a presequence of a first yeast signal sequence, but a pro-region from a second yeast alpha-factor. (See e.g., PCT WO 89/02463).

Typically, terminators are regulatory sequences, such as polyadenylation and transcription termination sequences, located 3' or downstream of the stop codon of the coding sequences. Usually, the terminator of native host cell proteins are operable when attached 3' of a CNGC-15 or Myosin IXa polypeptide coding sequences. Examples are the *Saccharomyces cerevisiae* alpha-factor terminator and the baculovirus terminator. Further, viral terminators are also operable in certain host cells; for instance, the SV40 terminator is functional in CHO cells.

For convenience, selectable markers, an origin of replication, and homologous host cells sequences may optionally be included in an expression vector. A selectable marker can be used to screen for host cells that potentially contain the expression vector. Such markers may render the host cell immune to drugs such as ampicillin, chloramphenicol, erythromycin, neomycin, and tetracycline. Also, markers may be biosynthetic genes, such as those in the histidine, tryptophan, and leucine pathways. Thus, when leucine is absent from the media, for example, only the cells with a biosynthetic gene in the leucine pathway will survive.

An origin of replication may be needed for the expression vector to replicate in the host cell. Certain origins of replication enable an expression vector to be reproduced at a high copy number in the presence of the appropriate proteins within the cell. Examples of origins are the 2μ and

autonomously replicating sequences, which are effective in yeast; and the viral T-antigen, effective in COS-7 cells.

Expression vectors may be integrated into the host cell genome or remain autonomous within the cell. Polynucleotide sequences homologous to sequences within the host cell genome may be needed to integrate the expression cassette. The homologous sequences do not always need to be linked to the expression vector to be effective. For example, expression vectors can integrate into the CHO genome via an unattached dihydrofolate reductase gene. In yeast, it is more advantageous if the homologous sequences flank the expression cassette. Particularly useful homologous yeast genome sequences are those disclosed in PCT WO 90/01800, and the HIS4 gene sequences, described in Genbank, Accession No. J01331.

The choice of promoter, terminator, and other optional elements of an expression vector will also depend on the host cell chosen. The invention is not dependent on the host cell selected. Convenience and the level of protein expression will dictate the optimal host cell. A variety of hosts for expression are known in the art and available from the American Type Culture Collection (ATCC). Bacterial hosts suitable for expressing a CNGC-15 and/or Myosin IXa polypeptide include, without limitation: *Campylobacter, Bacillus, Escherichia, Lactobacillus, Pseudomonas, Staphylococcus*, and *Streptococcus*. Yeast hosts from the following genera may be utilized: *Candida, Hansenula, Kluyveromyces, Pichia, Saccharomyces, Schizosaccharomyces*, and *Yarrowia*. Immortalized mammalian host cells include but are not limited to CHO cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (e.g., Hep G2), and other cell lines. A number of insect cell hosts are also available for expression of heterologous proteins: *Aedes aegypti, Bombyx mori, Drosophila melanogaster*, and *Spodoptera frugiperda* (PCT WO 89/046699; Carbonell *et al.* (1985) *J. Virol. 56*:153; Wright *et al.* (1986) *Nature 321*:718; Smith *et al.* (1983) *Mol. Cell. Biol. 3*:2156; and see generally, Fraser *et al.* (1989) *In Vitro Cell. Dev. Biol. 25*:225).

Transformation

After vector construction, the desired CNGC-15 and/or Myosin IXa polypeptide expression vector is inserted into the host cell. Many transformation techniques exist for inserting expression vectors into bacterial, yeast, insect, and mammalian cells. The transformation procedure to introduce the expression vector depends upon the host to be transformed.

Methods of introducing exogenous DNA into bacterial hosts are well-known in the art, and typically protocol includes either treating the bacteria with $CaCl_2$ or other agents, such as divalent cations and DMSO. DNA can also be introduced into bacterial cells by electroporation or viral infection. Transformation procedures usually vary with the bacterial species to be transformed. See e.g. (Masson *et al.* (1989) *FEMS Microbiol. Lett. 60*:273; Palva *et al.* (1982) *Proc. Natl. Acad. Sci. USA 79*:5582; EP Publ. Nos. 036 259 and 063 953; PCT WO 84/04541, *Bacillus*), (Miller *et al.* (1988) *Proc. Natl. Acad. Sci. 85*:856; Wang *et al.* (1990) *J. Bacteriol. 172*:949, *Campylobacter*), (Cohen *et al.* (1973) *Proc. Natl. Acad. Sci. 69*:2110; Dower *et al.* (1988) *Nucleic Acids Res. 16*:6127; Kushner *et al.* (1978) "An Improved Method for Transformation of *Escherichia coli* with ColE1-derived plasmids in *Genetic Engineering: Proceedings of the International Symposium on Genetic Engineering* (eds. H.W. Boyer and S. Nicosia); Mandel *et al.* (1970) *J. Mol. Biol. 53*:159; Taketo *et al.* (1988) *Biochim. Biophys. Acta 949*:318; *Escherichia*), (Chassy *et al.* (1987) *FEMS Microbiol. Lett. 44*:173, *Lactobacillus*); (Fiedler *et al.* (1988) *Anal. Biochem. 170*:38, *Pseudomonas*); (Augustin *et al.* (1990) *FEMS Microbiol. Lett. 66*:203, *Staphylococcus*), Barany *et al.* (1980) *J. Bacteriol. 144*:698; Harlander *et al.* (1987) "Transformation of *Streptococcus lactis* by electroporation," in *Streptococcal Genetics* (ed. J. Ferretti and R. Curtiss, III); Perry *et al.* (1981) *Infec. Immun. 32*:1295; Powell *et al.* (1988) *Appl. Environ. Microbiol. 54*:655; Somkuti *et al.* (1987) *Proc. 4th Evr. Cong. Biotechnology 1*:412, *Streptococcus*).

Transformation methods for yeast hosts are well-known in the art, and typically include either the transformation of spheroplasts or of intact yeast cells treated with alkali cations. Electroporation is another means for

transforming yeast hosts. See for example, *Methods in Enzymology*, Volume 194, 1991, "Guide to Yeast Genetics and Molecular Biology." Transformation procedures usually vary with the yeast species to be transformed. See e.g. (Kurtz *et al.* (1986) *Mol. Cell. Biol. 6*:142; Kunze *et al.* (1985) *J. Basic Microbiol. 25*:141, *Candida*); (Gleeson *et al.* (1986) *J. Gen. Microbiol. 132*:3459; Roggenkamp *et al.* (1986) *Mol. Gen. Genet. 202*:302, *Hansenula*); (Das *et al.* (1984) *J. Bacteriol. 158*:1165; De Louvencourt *et al.* (1983) *J. Bacteriol. 154*:1165; Van den Berg *et al.* (1990) *Biotechnology 8*:135, *Kluyveromyces*); (Cregg *et al.* (1985) *Mol. Cell.* Biol. 5:3376; Kunze *et al.* (1985) *J. Basic Microbiol. 25*:141; U.S. Patent Nos. 4,837,148 and 4,929,955, *Pichia*); (Hinnen *et al.* (1978) *Proc. Natl. Acad. Sci. USA 75*:1929; Ito *et al.* (1983) *J. Bacteriol. 153*:163, *Saccharomyces*); (Beach and Nurse (1981) *Nature 300*:706, *Schizosaccharomyces*); (Davidow *et al.* (1985) *Curr. Genet. 10*:39; Gaillardin *et al.* (1985) *Curr. Genet. 10*:49, *Yarrowia*).

Methods for introducing heterologous polynucleotides into mammalian cells are known in the art and include viral infection, dextran-mediated transfection, calcium phosphate precipitation, microparticle bombardment, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei.

The method for construction of an expression vector for transformation of insect cells for expression of recombinant herein is slightly different than that generally applicable to the construction of a bacterial expression vector, a yeast expression vector, or a mammalian expression vector. In an embodiment of the present invention, a baculovirus vector is constructed in accordance with techniques that are known in the art, for example, as described in Kitts *et al.* (1993) *Biotechniques 14*:810-817; Smith *et al.* (1983) *Mol. Cell. Biol. 3*:2156; and Luckow and Summer (1989) *Virol. 17*:31. In one embodiment of the present invention, a baculovirus expression vector is constructed substantially in accordance to Summers and Smith, Texas Agricultural Experiment Station Bulletin No. 1555 (1987). Moreover, materials and methods for baculovirus/insect cell expression systems are commercially available in kit form, for example, the MaxBac kit from Invitrogen (San Diego, CA).

Also, methods for introducing heterologous DNA into an insect host cell are known in the art. For example, an insect cell can be infected with a virus containing a CNGC-15 or Myosin IXa polypeptide coding sequence. When the virus is replicating in the infected cell, the CNGC-15 or Myosin IXa polypeptide will be expressed if operably linked to a suitable promoter. A variety of suitable insect cells and viruses are known and include following without limitation.

Insect cells from any order of the Class Insecta can be used to express the polypeptide of this invention. The orders Diptera and Lepidoptera are preferred. Example of insect species are listed in Weiss *et al.* "Cell Culture Methods for Large-Scale Propagation of Baculoviruses," in Granados *et al.* (eds., *The Biology of Baculoviruses: Vol. II Practical Application for Insect Control*, pp. 63-87 at p. 64 (1987). Insect cell lines derived from the following insects are exemplary: *Carpocapsa pomeonella* (preferably, cell line CP-128); *Trichoplusia ni* (preferably, cell line TN-368); *Autograph californica; Spodoptera frugiperda* (preferably, cell line Sf9); *Lymantria dispar; Mamestra brassicae; Aedes albopictus; Orgyia pseudotsugata; Neodiprio sertifer; Aedes aegypti; Antherae eucalypti; Gnorimoschema operceullela; Galleria mellonella; Spodoptera littolaris; Blatella germanic; Drosophila melanogaster: Heliothis zea; Spodoptera exigua; Rachiplusia ou; Plodia interpunctella; Amsaeta moorei; Agrotis c-nigrum, Adoxophyes orana; Agrotis segetum; Bombyx mori; Hyponomeuta malinellu; Colias eurytheme; Anticarsia germmetalia; Apanteles melanoscelu; Arctia caja;* and *Porthetria dispar*. Preferred insect cell lines are from *Spodoptera frugiperda*, and especially preferred is cell line Sf9. The Sf9 cell line used in the examples herein was obtained from Max D. Summers (Texas A & M University, College Station, Texas, 77843, USA). Other *S. frugiperda* cell lines, such as IPL-Sf-21AE III, are described in Vaughn *et al.* (1977) *In Vitro 13*:213-217.

The insect cell lines of this invention are suitable for the reproduction of numerous insect-pathogenic viruses such as parvoviruses, pox viruses, baculoviruses and rhabdoviruses, of which nucleopolyhedrosis viruses (NPV) and granulosis viruses (GV) from the group of baculoviruses are preferred.

Further preferred are NPV viruses such as those from *Autographa* spp., *Spodoptera* spp., *Trichoplusia* spp., *Rachiplusia* spp., *Gallerai* spp., and *Lymantria* spp. More preferred are baculovirus strain *Autographa californica* NPV (AcNPV), *Rachiplusia ou* NPV, *Galleria mellonella* NPV, and any plaque purified strains of AcNPV, such as E2, R9, S1, M3, characterized and described by Smith *et al.* (1978) *Virol. 89*:517-527.

Typically, insect cells *Spodoptera frugiperda* type 9 (SF9) are infected with baculovirus strain *Autographa californica* NPV (AcNPV) containing a CNGC-15 or Myosin IXa polypeptide coding sequence. Such a baculovirus is produced by homologous recombination between a transfer vector containing the coding sequence and baculovirus sequences and a genomic baculovirus DNA. Preferably, the genomic baculovirus DNA is linearized and contains a dysfunctional essential gene. The transfer vector, preferably, contains the nucleotide sequences needed to restore the dysfunctional gene and a baculovirus polyhedrin promoter and terminator operably linked to the CNGC-15 or Myosin IXa coding sequence. (See Kitts *et al.* (1993) *Biotechniques 14(5)*:810-817.

The transfer vector and linearized baculovirus genome are transfected into SF9 insect cells, and the resulting viruses probably containing the desired coding sequence. Without a functional essential gene the baculovirus genome cannot produce a viable virus. Thus, the viable viruses from the transfection most likely contain the CNGC-15 or Myosin IXa polypeptide coding sequence and the needed essential gene sequences from the transfer vector. Further, lack of occlusion bodies in the infected cells are another verification that the CNGC-15 or Myosin IXa polypeptide coding sequence was incorporated into the baculovirus genome.

The essential gene and the polyhedrin gene flank each other in the baculovirus genome. The coding sequence in the transfer vector is flanked at its 5′ with the essential gene sequences and the polyhedrin promoter and at its 3′ with the polyhedrin terminator. Thus, when the desired recombination event occurs the CNGC-15 or Myosin IXa polypeptide coding sequence displaces the baculovirus polyhedrin gene. Such baculoviruses without a polyhedrin gene will not produce occlusion bodies in the infected cells. Of

course, another means for determining if coding sequence was incorporated into the baculovirus genome is to sequence the recombinant baculovirus genomic DNA. Alternatively, expression of the desired CNGC-15 or Myosin IXa polypeptide by cells infected with the recombinant baculovirus is another verification means.

Monitoring CNGC-15 Polypeptide Expression Levels

Immunoassays and ligand binding assays can be utilized to confirm that the transformed host cell is expressing the desired CNGC-15 and/or Myosin IXa polypeptide.

For example, an immunofluorescence assay can be performed on transformed host cells without separating the CNGC-15 or Myosin IXa polypeptides from the cell. The host cells are first fixed onto a solid support, such as a microscope slide or microtiter well. This fixing step permeabilizes the cell membrane. Next, the fixed host cells are exposed to an anti-CNGC-15 or anti-Myosin IXa polypeptide antibody. Preferably, to increase the sensitivity of the assay, the fixed cells are exposed to a second antibody, which is labeled and binds to the anti-CNGC-15 or anti-Myosin IXa polypeptide antibody. Typically, the secondary antibody is labeled with an fluorescent marker. The host cells which express the CNGC-15 or Myosin IXa polypeptides will be fluorescently labeled and easily visualized under the microscope. See, for example, Hashido *et al.* (1992) *Biochem & Biophys. Res. Comm. 187(3)*:1241-1248.

Also, the CNGC-15 and Myosin IXa polypeptides do not need to be separated from the cell membrane for *in vitro* assays. The host cells may be fixed to a solid support, such as a microtiter plate. Alternatively, a crude membrane fraction can be separated from lysed host cells by centrifugation (See Adachi *et al.* (1992) *FEBS Lett 311(2)*:179-183. The fixed host cells or the crude membrane fraction is exposed to labeled ligand or ion. Typically, the ligand is labeled with radioactive atoms. The host cells which express the desired CNGC-15 or Myosin IXa polypeptide will bind with the labeled ligand which can be easily detected.

Purification

The purified CNGC-15 and Myosin IXa polypeptides are useful as compositions, for assays, and to produce antibodies.

CNGC-15 and Myosin IXa polypeptides can be isolated by a variety of steps including, for example, anion exchange chromatography, size exclusion chromatography, hydroxyapatite chromatography, hydrophobic interaction chromatography, metal chelation chromatography, reverse phase HPLC, affinity chromatography, and further ammonium sulfate precipitations. These techniques are well known to those of skill in the art.

For ligand binding studies, patch clamp analysis or other *in vitro* assays, the crude cell membrane fractions can be utilized. These membrane extracts can be isolated from cells which expressed CNGC-15 or Myosin IXa polypeptides by lysing the cells. Alternatively, whole cells, expressing CNGC-15 and/or Myosin IXa polypeptides, can be cultured in a microtiter plate.

Antibodies

Antibodies against CNGC-15 and Myosin IXa polypeptides are useful for affinity chromatography, immunofluorescent assays, and distinguishing CNGC-15 and Myosin IXa polypeptides.

Antibodies to the proteins of the invention, both polyclonal and monoclonal, may be prepared by conventional methods known to those skilled in the art. For example, monoclonal antibodies are prepared using the method of Kohler *et al.* (1975) *Nature 256*:495-496, or a modification thereof.

If desired, the antibodies (whether polyclonal or monoclonal) may be labeled using conventional techniques. Suitable labels include fluorophores, chromophores, radioactive atoms (particularly ^{32}P and ^{125}I), electron-dense reagents, enzymes, and ligands having specific binding partners. Enzymes are typically detected by their activity. For example, horseradish peroxidase is usually detected by its ability to convert 3,3',5,5'-tetra-methylbenzidine (TMB) to a blue pigment, quantifiable with a spectrophotometer. "Specific

binding partner" refers to a protein capable of binding a ligand molecule with high specificity, as for example in the case of an antigen and a monoclonal antibody specific therefor. Other specific binding partners include biotin and avidin or streptavidin, IgG and protein A, and the numerous receptor-ligand couples known in the art. It should be understood that the above description is not meant to categorize the various labels into distinct classes, as the same label may serve in several different modes. For example, ^{125}I may serve as a radioactive label or as an electron-dense reagent. HRP may serve as enzyme or as antigen for a MAb. Further, one may combine various labels for desired effect. For example, MAbs and avidin also require labels in the practice of this invention: thus, one might label a MAb with biotin, and detect its presence with avidin labeled with ^{125}I, or with an anti-biotin MAb labeled with HRP. Other permutations and possibilities will be readily apparent to those of ordinary skill in the art, and are considered as equivalents within the scope of the instant invention.

Screening for Agonists and Antagonists

CNGC-15 and Myosin IXa polypeptides can also be used to screen combinatorial libraries to identify agonist or antagonists. For example, a "library" of peptides may be synthesized following the methods disclosed in U.S. Patent No. 5,010,175, and in PCT WO 91/17823, both incorporated herein by reference in full. The peptide library is first screened for binding to the selected CNGC-15 or Myosin IXa polypeptide. The peptides are then tested for their ability to inhibit or enhance CNGC-15 or Myosin IXa activity. Peptides exhibiting the desired activity are then isolated and sequenced.

CNGC-15 and Myosin IXa agonists or antagonists may be screened using any available method. The assay conditions ideally should resemble the conditions under which the CNGC-15 or Myosin IXa activity is exhibited *in vivo*, i.e., under physiologic pH, temperature, ionic strength, etc. Suitable agonists or antagonists will exhibit strong inhibition or enhancement of the CNGC-15 or Myosin IXa activity at concentrations which do not raise toxic side effects in the subject. Agonists or antagonists which compete for binding to the CNGC-15 or Myosin IXa polypeptide may require concentrations equal

to or greater than the native CNGC-15 or Myosin IXa concentration, while inhibitors capable of binding irreversibly to the polypeptide may be added in concentrations on the order of the native CNGC-15 or Myosin IXa concentration.

Signal Transduction Assays

Most cellular Ca^{2+} ions are sequestered in the mitochondria, endoplasmic reticulum, and other cytoplasmic vesicles, but binding of endothelin to receptor will trigger the increase of free Ca^{2+} ions in the cytoplasm. With fluorescent dyes, such as *fura*-2, the concentration of free Ca^{2+} can be monitored. The ester of fura-2 is added to the media of the host cells expressing receptor polypeptides. The ester of fura-2 is lipophilic and diffuses across the membrane. Once inside the cell, the fura-2 ester is hydrolyzed by cytosolic esterases to its non-lipophilic form, and then the dye cannot diffuse back out of the cell. The non-lipophilic form of fura-2 will fluoresce when it binds to the free Ca^{2+} ions, which are released after binding of a ligand to the receptor. The fluorescence can be measured without lysing the cells at an excitation spectrum of 340 nm and at fluorescence spectrum of 500 nm. See Sakurai *et al.* EP 480 381 and Adachi *et al.* (1992) *FEBS Lett 311(2)*:179-183 for examples of assays measuring free intracellular Ca^{2+} concentrations.

The rise of free cytosolic Ca^{2+} concentrations is preceded by the hydrolysis of phosphatidylinositol 4,5-bisphosphate. Hydrolysis of this phospholipid by the plasma-membrane enzyme phospholipase C yields 1,2-diacylglycerol (DAG), which remains in the membrane, and the water-soluble inosital 1,4,5-trisphosphate (IP_3). Binding of endothelin or endothelin agonists will increase the concentration of DAG and IP_3. Thus, signal transduction activity can be measured by monitoring the concentration of these hydrolysis products.

To measure the IP_3 concentrations, radioactively labeled ^3H-inositol is added to the media of host cells expressing CNGC-15 or Myosin IXa polypeptides. The ^3H-inositol taken up by the cells and after stimulation of the cells with endothelin or endothelin agonist, the resulting inositol triphosphate

is separated from the mono and di-phosphate forms and measured. See Sakurai *et al.*, EP 480 381. Alternatively, Amersham provides an inosital 1,4,5-trisphosphate assay system. With this system Amersham provides tritylated inosital 1,4,5-trisphosphate and a receptor capable of distinguishing the radioactive inositol from other inositol phosphates. With these reagents an effective and accurate competition assay can be performed to determine the inositol triphosphate levels.

Pharmaceutical Compositions

Pharmaceutical compositions can comprise either polypeptides, antibodies, or polynucleotides of the claimed invention. The pharmaceutical compositions will comprise a therapeutically effective amount of either polypeptides, antibodies, or polynucleotides of the claimed invention.

The term "therapeutically effective amount" as used herein refers to an amount of a therapeutic agent to treat, ameliorate, or prevent a desired disease or condition, or to exhibit a detectable therapeutic or preventative effect. The effect can be detected by, for example, chemical markers or antigen levels. Therapeutic effects also include reduction in physical symptoms, such as decreased body temperature. The precise effective amount for a subject will depend upon the subject's size and health, the nature and extent of the condition, and the therapeutics or combination of therapeutics selected for administration. Thus, it is not useful to specify an exact effective amount in advance. However, the effective amount for a given situation can be determined by routine experimentation and is within the judgment of the clinician.

For purposes of the present invention, an effective dose will be from about 0.01 mg/kg to 50 mg/kg or 0.05 mg/kg to about 10 mg/kg of the polypeptide or DNA construct in the individual to which it is administered

A pharmaceutical composition can also contain a pharmaceutically acceptable carrier. The term "pharmaceutically acceptable carrier" refers to a carrier for administration of a therapeutic agent, such as antibodies or a polypeptide, genes, and other therapeutic agents. The term refers to any pharmaceutical carrier that does not itself induce the production of antibodies

harmful to the individual receiving the composition, and which may be administered without undue toxicity. Suitable carriers may be large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, and inactive virus particles. Such carriers are well known to those of ordinary skill in the art.

Pharmaceutically acceptable salts can be used therein, for example, mineral acid salts such as hydrochlorides, hydrobromides, phosphates, sulfates, and the like; and the salts of organic acids such as acetates, propionates, malonates, benzoates, and the like. A thorough discussion of pharmaceutically acceptable excipients is available in *Remington's Pharmaceutical Sciences* (Mack Pub. Co., N.J. 1991).

Pharmaceutically acceptable carriers in therapeutic compositions may contain liquids such as water, saline, glycerol and ethanol. Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present in such vehicles. Typically, the therapeutic compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection may also be prepared. Liposomes are included within the definition of a pharmaceutically acceptable carrier.

Delivery Methods

Once formulated, the compositions of the invention can be administered directly to the subject. The subjects to be treated can be mammals or birds. In particular, human subjects can be treated.

Direct delivery of the compositions will generally be accomplished by injection, either subcutaneously, intraperitoneally, intravenously or intramuscularly or delivered to the interstitial space of a tissue. The compositions can also be administered into a tumor or lesion. Other modes of administration include oral and pulmonary administration, suppositories, and transdermal applications, needles, and gene guns or hyposprays. Dosage treatment may be a single dose schedule or a multiple dose schedule.

Alternatively, the Myosin IXa or CNGC-15 polypeptides could be stably expressed in an organ of a mammal, and then the organ could be xenografted into a human in need of such treatment.

Gene Delivery Vehicles

Gene therapy vehicles for delivery of constructs including a coding sequence of a therapeutic of the invention, to be delivered to the mammal for expression in the mammal, can be administered either locally or systemically. These constructs can utilize viral or non-viral vector approaches in *in vivo* or *ex vivo* modality. Expression of such coding sequence can be induced using endogenous mammalian or heterologous promoters. Expression of the coding sequence *in vivo* can be either constitutive or regulated.

The invention includes gene delivery vehicles capable of expressing the contemplated nucleic acid sequences. The gene delivery vehicle is preferably a viral vector and, more preferably, a retroviral, adenoviral, adeno-associated viral (AAV), herpes viral, or alphavirus vector. The viral vector can also be an astrovirus, coronavirus, orthomyxovirus, papovavirus, paramyxovirus, parvovirus, picornavirus, poxvirus, or togavirus viral vector. See generally, Jolly *et al.* (1994) *Cancer Gene Therapy 1*:51-64; Kimura *et al.* (1994) *Human Gene Therapy 5*:845-852; Connelly *et al.* (1995) *Human Gene Therapy 6*:185-193; and Kaplitt *et al.* (1994) *Nature Genetics 6*:148-153.

Retroviral vectors are well known in the art and we contemplate that any retroviral gene therapy vector is employable in the invention, including B, C and D type retroviruses, xenotropic retroviruses (for example, NZB-X1, NZB-X2 and NZB9-1 (see O'Neill (1985) *J. Vir. 53*:160) polytropic retroviruses (for example, MCF and MCF-MLV (see Kelly *et al.* (1983) *J. Vir. 45*:291), spumaviruses and lentiviruses. See *RNA Tumor Viruses*, Second Edition, Cold Spring Harbor Laboratory, 1985.

Portions of the retroviral gene therapy vector may be derived from different retroviruses. For example, retrovector LTRs may be derived from a Murine Sarcoma Virus, a tRNA binding site from a Rous Sarcoma Virus, a packaging signal from a Murine Leukemia Virus, and an origin of second strand synthesis from an Avian Leukosis Virus.

These recombinant retroviral vectors may be used to generate transduction competent retroviral vector particles by introducing them into appropriate packaging cell lines (see US Serial No. 07/800,921, filed November 29, 1991). Retrovirus vectors can be constructed for site-specific integration into host cell DNA by incorporation of a chimeric integrase enzyme into the retroviral particle. See, US Serial No. 08/445,466 filed May 22, 1995. It is preferable but not required that the recombinant viral vector is a replication defective recombinant virus.

Packaging cell lines suitable for use with the above-described retrovirus vectors are well known in the art, are readily prepared (see US Serial No. 08/240,030, filed May 9, 1994; see also WO 92/05266), and can be used to create producer cell lines (also termed vector cell lines or "VCLs") for the production of recombinant vector particles. Preferably, the packaging cell lines are made from human parent cells (e.g., HT1080 cells) or mink parent cell lines, which eliminates inactivation in human serum.

Preferred retroviruses for the construction of retroviral gene therapy vectors include Avian Leukosis Virus, Bovine Leukemia, Virus, Murine Leukemia Virus, Mink-Cell Focus-Inducing Virus, Murine Sarcoma Virus, Reticuloendotheliosis Virus and Rous Sarcoma Virus. Particularly preferred Murine Leukemia Viruses include 4070A and 1504A (Hartley *et al.* (1976) *J. Virol. 19*:19-25), Abelson (ATCC No. VR-999), Friend (ATCC No. VR-245), Graffi, Gross (ATCC Nol VR-590), Kirsten, Harvey Sarcoma Virus and Rauscher (ATCC No. VR-998) and Moloney Murine Leukemia Virus (ATCC No. VR-190). Such retroviruses may be obtained from depositories or collections such as the American Type Culture Collection ("ATCC") in Rockville, Maryland or isolated from known sources using commonly available techniques.

Exemplary known retroviral gene therapy vectors employable in this invention include those described in GB 2200651, EP 0415731, EP 0345242, WO 89/02468, WO 89/05349, WO 89/09271, WO 90/02806, WO 90/07936, WO 94/03622, WO 93/25698, WO 93/25234, WO 93/11230, WO 93/10218, WO 91/02805, in U.S. Patent No. 5,219,740, No. 4,405,712, No. 4,861,719,

No. 4,980,289 and No. 4,777,127, in U.S. Serial No. 07/800,921 and in Vile *et al.* (1993) *Cancer Res. 53*:3860-3864; Vile *et al.* (1993) *Cancer Res. 53*:962-967; Ram *et al.* (1993) *Cancer Res. 53*:83-88; Takamiya *et al.* (1992) *J. Neurosci. Res. 33*:493-503; Baba *et al.* (1993) *J. Neurosurg. 79*:729-735; Mann *et al.* (1983) *Cell 33*:153; Cane *et al.* (1984) *Proc. Natl. Acad. Sci. 81*:6349; and Miller *et al.* (1990) *Human Gene Therapy 1*.

Human adenoviral gene therapy vectors are also known in the art and employable in this invention. See, for example, Berkner *et al.* (1988) *Biotechniques 6*:616; Rosenfeld *et al.* (1991) *Science 252*:431; and WO 93/07283, WO 93/06223, and WO 93/07282. Exemplary known adenoviral gene therapy vectors employable in this invention include those described in the above referenced documents and in WO 94/12649, WO 93/03769, WO 93/19191, WO 94/28938, WO 95/11984, WO 95/00655, WO 95/27071, WO 95/29993, WO 95/34671, WO 96/05320, WO 94/08026, WO 94/11506, WO 93/06223, WO 94/24299, WO 95/14102, WO 95/24297, WO 95/02697, WO 94/28152, WO 94/24299, WO 95/09241, WO 95/25807, WO 95/05835, WO 94/18922, WO 95/09654. Alternatively, administration of DNA linked to killed adenovirus as described in Curiel *et al.* (1992) *Hum. Gene Ther. 3*:147-154 may be employed.

The gene delivery vehicles of the invention also include adenovirus associated virus (AAV) vectors. Leading and preferred examples of such vectors for use in this invention are the AAV-2 based vectors disclosed in Srivastava, WO 93/09239. Most preferred AAV vectors comprise the two AAV inverted terminal repeats in which the native D-sequences are modified by substitution of nucleotides, such that at least about 5 native nucleotides and up to 18 native nucleotides, preferably at least about 10 native nucleotides up to 18 native nucleotides, most preferably 10 native nucleotides are retained and the remaining nucleotides of the D-sequence are deleted or replaced with non-native nucleotides. The native D-sequences of the AAV inverted terminal repeats are sequences of 20 consecutive nucleotides in each AAV inverted terminal repeat (i.e., there is one sequence at each end) which are not involved in HP formation. The non-native replacement nucleotide may be any nucleotide other than the nucleotide found in the native D-sequence in the

same position. Other employable exemplary AAV vectors are pWP-19, pWN-1, both of which are disclosed in Nahreini *et al.* (1993) *Gene 124*:257-262. Another example of such an AAV vector is psub201. See Samulski *et al.* (1987) *J. Virol. 61*:3096. Another exemplary AAV vector is the Double-D ITR vector. Construction of the Double D ITR vector is disclosed in U.S. Patent No. 5,478,745. Still other vectors are those disclosed in Carter, U.S. Patent No. 4,797,368 and Muzyczka, U.S. Patent No. 5,139,941, Chartejee, U.S. Patent No. 5,474,935, and Kotin, PCT Patent Publication WO 94/288157. Yet a further example of an AAV vector employable in this invention is SSV9AFABTKneo, which contains the AFP enhancer and albumin promoter and directs expression predominantly in the liver. Its structure and construction are disclosed in SU, (1996) *Human Gene Therapy 7*:463-470. Additional AAV gene therapy vectors are described in U.S. 5,354,678, U.S. 5,173,414, U.S. 5,139,941, and U.S. 5,252,479.

The gene therapy vectors of the invention also include herpes vectors. Leading and preferred examples are herpes simplex virus vectors containing a sequence encoding a thymidine kinase polypeptide such as those disclosed in U.S. 5,288,641 and EP 0176170 (Roizman). Additional exemplary herpes simplex virus vectors include HFEM/ICP6-LacZ disclosed in WO 95/04139 (Wistar Institute), pHSVlac described in Geller *et al.* (1988) *Science 241*:1667-1669 and in WO 90/09441 and WO 92/07945, HSV Us3::pgC-lacZ described in Fink, (1992) *Human Gene Therapy 3*:11-19 and HSV 7134, 2 RH 105 and GAL4 described in EP 0453242 (Breakefield), and those deposited with the ATCC as accession numbers ATCC VR-977 and ATCC VR-260.

Also contemplated are alpha virus gene therapy vectors that can be employed in this invention. Preferred alpha virus vectors are Sindbis viruses vectors. Togaviruses, Semliki Forest virus (ATCC VR-67; ATCC VR-1247), Middleberg virus (ATCC VR-370), Ross River virus (ATCC VR-373; ATCC VR-1246), Venezuelan equine encephalitis virus (ATCC VR923; ATCC VR-1250; ATCC VR-1249; ATCC VR-532), and those described in U.S. patents 5,091,309, 5,217,879, and WO 92/10578, WO 95/07994, U.S. 5,091,309 and U.S. 5,217,879 are employable. Such alpha viruses may be obtained from depositories or collections such as the ATCC in Rockville, Maryland or

isolated from known sources using commonly available techniques. Preferably, alphavirus vectors with reduced cytotoxicity are used (see co-owned U.S.S.N. 08/679640).

DNA vector systems such as eukaryotic layered expression systems are also useful for expressing the nucleic acids of the invention. See WO 95/07994 for a detailed description of eukaryotic layered expression systems. Preferably, the eukaryotic layered expression systems of the invention are derived from alphavirus vectors and most preferably from Sindbis viral vectors.

Other viral vectors suitable for use in the present invention include those derived from poliovirus, for example ATCC VR-58 and those described in Evans *et al.* (1989) *Nature 339*:385 and Sabin *et al.* (1973) *J. Biol. Standardization 1*:115; rhinovirus, for example ATCC VR-1110 and those described in Arnold *et al.* (1990) *J. Cell Biochem* L401; pox viruses such as canary pox virus or vaccinia virus, for example ATCC VR-111 and ATCC VR-2010 and those described in Fisher-Hoch *et al.* (1989) *Proc. Natl. Acad. Sci. 86*:317; Flexner *et al.* (1989) *Ann. NY Acad. Sci. 569*:86; Flexner *et al.* (1990) *Vaccine 8*:17; in U.S. 4,603,112 and U.S. 4,769,330 and in WO 89/01973; SV40 virus, for example ATCC VR-305 and those described in Mulligan *et al.* (1979) *Nature 277*:108 and Madzak *et al.* (1992) *J. Gen. Vir. 73*:1533; influenza virus, for example ATCC VR-797 and recombinant influenza viruses made employing reverse genetics techniques as described in U.S. 5,166,057 and in Enami *et al.* (1990) *Proc. Natl. Acad. Sci. 87*:3802-3805, Enami *et al.* (1991) *J. Virol. 65*:2711-2713; Luytjes *et al.* (1989) *Cell 59*:110 (see also McMicheal, (1983) *NE J. Med. 309*:13 and Yap *et al.* (1978) *Nature 273*:238 and Yap *et al.* (1979) *Nature 277*:108); human immunodeficiency virus as described in EP 0386882 and in Buchschacher *et al.* (1992) *J. Vir. 66*:2731; measles virus, for example ATCC VR-67 and VR-1247 and those described in EP 0440219; Aura virus, for example ATCC VR-368; Bebaru virus, for example ATCC VR-600 and ATCC VR-1240; Cabassou virus, for example ATCC VR-922; Chikungunya virus, for example ATCC VR-64 and ATCC VR-1241; Fort Morgan Virus, for example ATCC VR-924; Getah virus, for example ATCC VR-369 and ATCC VR-1243;

Kyzylagach virus, for example ATCC VR-927; Mayaro virus, for example ATCC VR-66; Mucambo virus, for example ATCC VR-580 and ATCC VR-1244; Ndumu virus, for example ATCC VR-371; Pixuna virus, for example ATCC VR-372 and ATCC VR-1245; Tonate virus, for example ATCC VR-925; Triniti virus, for example ATCC VR-469; Una virus, for example ATCC VR-374; Whataroa virus, for example ATCC VR-926; Y-62-33 virus, for example ATCC VR-375; O'Nyong virus, Eastern encephalitis virus, for example ATCC VR-65f and ATCC VR-1242; Western encephalitis virus, for example ATCC VR-70, ATCC VR-1251, ATCC VR-622 and ATCC VR-1252; and coronavirus, for example ATCC VR-740 and those described in Hamre *et al.* (1966) *Proc. Soc. Exp. Biol. Med. 121*:190.

Delivery of the compositions of this invention into cells is not limited to the above mentioned viral vectors. Other delivery methods and media may be employed such as, for example, nucleic acid expression vectors, polycationic condensed DNA linked or unlinked to killed adenovirus alone, for example see U.S. Serial No. 08/366,787, filed December 30, 1994 and Curiel *et al.* (1989) *Hum. Gene Ther. 3*:147-154; ligand linked DNA, for example see Wu *et al.* (1989) *J. Biol. Chem. 264*:16985-16987, eucaryotic cell delivery vehicles cells, for example see U.S. Serial No. 08/240,030, filed May 9, 1994, and U.S. Serial No. 08/404,796, deposition of photopolymerized hydrogel materials, hand-held gene transfer particle gun, as described in U. S. Patent No. 5,149,655, ionizing radiation as described in U.S. 5,206,152 and in WO 92/11033, nucleic charge neutralization or fusion with cell membranes. Additional approaches are described in Philip *et al.* (1994) *Mol. Cell Biol. 14*:2411-2418 and in Woffendin *et al.* (1994) *Proc. Natl. Acad. Sci. 91*:1581-1585.

Particle mediated gene transfer may be employed, for example see U.S. Serial No. 60/023,867. Briefly, the sequence can be inserted into conventional vectors that contain conventional control sequences for high level expression, and then incubated with synthetic gene transfer molecules such as polymeric DNA-binding cations like polylysine, protamine, and albumin, linked to cell targeting ligands such as asialoorosomucoid, as described in Wu *et al.* (1987) *J. Biol. Chem. 262*:4429-4432, insulin as

described in Hucked, (1990) *Biochem. Pharmacol. 40*:253-263, galactose as described in Plank *et al.* (1992) *Bioconjugate Chem. 3*:533-539, lactose or transferrin.

Naked DNA may also be employed. Exemplary naked DNA introduction methods are described in WO 90/11092 and U.S. 5,580,859. Uptake efficiency may be improved using biodegradable latex beads. DNA coated latex beads are efficiently transported into cells after endocytosis initiation by the beads. The method may be improved further by treatment of the beads to increase hydrophobicity and thereby facilitate disruption of the endosome and release of the DNA into the cytoplasm.

Liposomes that can act as gene delivery vehicles are described in U.S. 5,422,120, WO 95/13796, WO 94/23697, WO 91/144445 and EP 524,968. As described in co-owned U.S. Serial No. 60/023,867, on non-viral delivery, the nucleic acid sequences encoding a polypeptide can be inserted into conventional vectors that contain conventional control sequences for high level expression, and then be incubated with synthetic gene transfer molecules such as polymeric DNA-binding cations like polylysine, protamine, and albumin, linked to cell targeting ligands such as asialoorosomucoid, insulin, galactose, lactose, or transferrin. Other delivery systems include the use of liposomes to encapsulate DNA comprising the gene under the control of a variety of tissue-specific or ubiquitously-active promoters. Further non-viral delivery suitable for use includes mechanical delivery systems such as the approach described in Woffendin *et al.* (1994) *Proc. Natl. Acad. Sci. U.S.A. 91 (24)*:11581-11585. Moreover, the coding sequence and the product of expression of such can be delivered through deposition of photopolymerized hydrogel materials. Other conventional methods for gene delivery that can be used for delivery of the coding sequence include, for example, use of hand-held gene transfer particle gun, as described in U.S. 5,149,655; use of ionizing radiation for activating transferred gene, as described in U.S. 5,206,152 and WO 92/11033.

Exemplary liposome and polycationic gene delivery vehicles are those described in U.S. 5,422,120 and 4,762,915; in WO 95/13796; WO 94/23697; and WO 91/14445; in EP 0524968; and in Stryer *et al.* (1975) *Biochemistry*,

pp. 236-240, W. H. Freeman, San Francisco; Szoka *et al.* (1980) *Biochim. Biophys. Acta. 600*:1; Bayer, (1979) *Biochim. Biophys. Acta. 550*:464; Rivnay *et al.* (1987) *Meth. Enzymol. 149*:119; Wang *et al.* (1987) *Proc. Natl. Acad. Sci. 84*:7851; Plant (1989) *Anal. Biochem. 176*:420.

Pharmaceutical Compositions

In addition to the pharmaceutically acceptable carriers and salts described above, the following additional agents can be used with polynucleotide compositions.

A. Polypeptides

One example is polypeptides which include, without limitation: asioloorosomucoid (ASOR); transferrin; asialoglycoproteins; antibodies; antibody fragments; ferritin; interleukins; interferons, granulocyte, macrophage colony stimulating factor (M-CSF), stem cell factor and erythropoietin. Viral antigens, such as envelope proteins, can also be used. Also, proteins from other invasive organisms, such as the 17 amino acid peptide from the circumsporozoite protein of plasmodium falciparum known as RII.

B. Hormones, Vitamins, Etc.

Other groups that can be included are, for example: hormones, steroids, androgens, estrogens, thyroid hormone, or vitamins, folic acid.

C. Polyalkylenes, Polysaccharides, Etc.

Also, polyalkylene glycol can be included with the desired polynucleotides. In a preferred embodiment, the polyalkylene glycol is polyethylene glycol. In addition, mono-, di-, or polysaccarides can be included. In a preferred embodiment of this aspect, the polysaccharide is dextran or DEAE-dextran. Also, chitosan and poly(lactide-co-glycolide)

D. Lipids, and Liposomes

The desired polynucleotide can also be encapsulated in lipids or packaged in liposomes prior to delivery to the subject or to cells derived therefrom.

Lipid encapsulation is generally accomplished using liposomes which are able to stably bind or entrap and retain nucleic acid. The ratio of condensed polynucleotide to lipid preparation can vary but will generally be around 1:1 (mg DNA:micromoles lipid), or more of lipid. For a review of the use of liposomes as carriers for delivery of nucleic acids, see, Hug *et al.* (1991) *Biochim. Biophys. Acta. 1097*:1-17; Straubinger *et al.* (1983) *Methods of Enzymology 101*:512-527.

Liposomal preparations for use in the instant invention include cationic (positively charged), anionic (negatively charged) and neutral preparations. Cationic liposomes have been shown to mediate intracellular delivery of plasmid DNA (Felgner *et al.* (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*:7413-7416); mRNA (Malone *et al.* (1989) *Proc. Natl. Acad. Sci. U.S.A. 86*:6077-6081); and purified transcription factors (Debs *et al.* 1990) *J. Biol. Chem. 265*:10189-10192), in functional form.

Cationic liposomes are readily available. For example, N[1-2,3-dioleyloxy)propyl]-N,N,N-triethylammonium (DOTMA) liposomes are available under the trademark Lipofectin, from GIBCO BRL, Grand Island, NY. (See, also, Felgner *et al.* (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*:7413-7416). Other commercially available liposomes include transfectace (DDAB/DOPE) and DOTAP/DOPE (Boerhinger). Other cationic liposomes can be prepared from readily available materials using techniques well known in the art. See, e.g., Szoka *et al.* (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*:4194-4198; PCT Publication No. WO 90/11092 for a description of the synthesis of DOTAP (1,2-bis(oleoyloxy)-3-(trimethylammonio)propane) liposomes.

Similarly, anionic and neutral liposomes are readily available, such as from Avanti Polar Lipids (Birmingham, AL), or can be easily prepared using readily available materials. Such materials include phosphatidyl choline, cholesterol, phosphatidyl ethanolamine, dioleoyfphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), dioleoylphoshatidyl ethanolamine (DOPE), among others. These materials can also be mixed with the DOTMA and DOTAP starting materials in appropriate ratios. Methods for making liposomes using these materials are well known in the art.

The liposomes can comprise multilammelar vesicles (MLVs), small unilamellar vesicles (SUVs), or large unilamellar vesicles (LUVs). The various liposome-nucleic acid complexes are prepared using methods known in the art. See, e.g., Straubinger *et al.* (1983) *Methods of Immunology 101*:512-527; Szoka *et al.* (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*:4194-4198; Papahadjopoulos *et al.* (1975) *Biochim. Biophys. Acta. 394*:483; Wilson *et al.* (1979) *Cell 17*:77; Deamer *et al.* (1976) *Biochim. Biophys. Acta. 443*:629; Ostro *et al.* (1977) *Biochem. Biophys. Res. Commun. 76*:836; Fraley *et al.* (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*:3348; Enoch *et al.* (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*:145; Fraley *et al.* (1980) *J. Biol. Chem. 255*:10431; Szoka *et al.* (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*:145; and Schaefer-Ridder *et al.* (1982) *Science 215*:166.

E. Liproproteins

In addition, lipoproteins can be included with the polynucleotide to be delivered. Examples of lipoproteins to be utilized include: chylomicrons, HDL, IDL, LDL, and VLDL. Mutants, fragments, or fusions of these proteins can also be used. Also, modifications of naturally occurring lipoproteins can be used, such as acetylated LDL. These lipoproteins can target the delivery of polynucleotides to cells expressing lipoprotein receptors. Preferably, if lipoproteins are included with the polynucleotide to be delivered, no other targeting ligand is included in the composition.

Naturally occurring lipoproteins comprises of a lipid and a protein portion. The protein portion is known as apoproteins. At the present, apoproteins A, B, C, D, and E have been isolated and identified. At least two of these contain several proteins, designated by Roman numerals, AI, AII, AIV, CI, CII, and CIII.

A lipoprotein can comprise more than one apoprotein. For example, naturally occurring chylomicrons comprises of A, B, C, and E; over time these lipoproteins lose A and acquire C and E apoproteins. VLDL comprises A, B, C, and E apoproteins; LDL comprises apoprotein B; and HDL comprises apoproteins A, C, and E.

The amino acids of these apoproteins are known and are described in, for example, Breslow (1985) *Annu. Rev. Biochem. 54*:699; Law *et al.* (1986) *Adv. Exp. Med. Biol. 151*:162; Chen *et al.* 1986) *J. Biol. Chem. 261*:12918; Kane *et al.* (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*:2465; and Utermann *et al.* (1984) *Hum. Genet. 65*:232.

Lipoproteins contain a variety of lipids including triglycerides, cholesterol (free and esters), and phospholipids. The composition of the lipids varies in naturally occurring lipoproteins. For example, chylomicrons comprise mainly triglycerides. A more detailed description of the lipid content of naturally occurring lipoproteins can be found, for example, in *Meth. Enzym. 128* (1986). The composition of the lipids are chosen to aid in conformation of the apoprotein for receptor binding activity. The composition of lipids can also be chosen to facilitate hydrophobic interaction and association with the polynucleotide binding molecule.

Naturally occurring lipoproteins can be isolated from serum by ultracentrifugation, for instance. Such methods are described in *Meth. Enzy., Supra*; Pitas *et al.* (1980) *J. Biochem. 255*:5454-5460; and Mahey *et al.* (1979) *J. Clin. Invest. 64*:743-750.

Lipoproteins can also be produced by *in vitro* or recombinant methods by expression of the apoprotein genes in a desired host cell. See, for example, Atkinson *et al.* (1986) *Annu. Rev. Biophys. Chem. 15*:403 and Radding *et al.* (1958) *Biochim. Biophys. Acta. 30*:443.

Lipoproteins can also be purchased from commercial suppliers, such as Biomedical Technologies, Inc., Stoughton, Massachusetts, U.S.A.

Further description of lipoproteins can be found in Zuckermann *et al.*, PCT. Appln. No. US 97/14465.

F. Polycationic Agents

Polycationic agents can be included, with or without lipoprotein, in a composition with the desired polynucleotide to be delivered.

Polycationic agents, typically exhibit a net positive charge at physiological relevant pH and are capable of neutralizing the electrical charge of nucleic acids to facilitate delivery to a desired location. These agents have

in vitro, *ex vivo*, and *in vivo* applications. Polycationic agents can be used to deliver nucleic acids to a living subject either intramuscularly, subcutaneously, etc.

The following are examples of useful polypeptides as polycationic agents: polylysine, polyarginine, polyornithine, and protamine. Other examples include histones, protamines, human serum albumin, DNA binding proteins, non-histone chromosomal proteins, coat proteins from DNA viruses, such as ϕX174, transcriptional factors also contain domains that bind DNA and therefore may be useful as nucleic acid condensing agents. Briefly, transcriptional factors such as C/CEBP, c-*jun*, c-*fos*, AP-1, AP-2, AP-3, CPF, Prot-1, Sp-1, Oct-1, Oct-2, CREP, and TFIID contain basic domains that bind DNA sequences.

Organic polycationic agents include: spermine, spermidine, and putrescine.

The dimensions and the physical properties of a polycationic agent can be extrapolated from the list above to construct other polypeptide polycationic agents or to produce synthetic polycationic agents.

Synthetic Polycationic Agents

Synthetic polycationic agents which are useful include, for example, DEAE-dextran, polybrene. Lipofectin, and lipofectAMINE are monomers that form polycationic complexes when combined with polynucleotides. See for example, Zuckermann *et al.*, PCT Appln. U.S. 97/14465.

A polynucleotide composition can comprises therapeutically effective amount of a gene therapy vehicle, as the term is defined above. For purposes of the present invention, an effective dose will be from about 0.01 mg/kg to 50 mg/kg or 0.05 mg/kg to about 10 mg/kg of the DNA constructs in the individual to which it is administered.

Delivery Methods

Once formulated, the polynucleotide compositions of the invention can be administered (1) directly to the subject; (2) delivered *ex vivo*, to cells derived from the subject; or (3) *in vitro* for expression of recombinant

proteins. The subjects to be treated can be mammals or birds. Also, human subjects can be treated.

Direct delivery of the compositions will generally be accomplished by injection, either subcutaneously, intraperitoneally, intravenously or intramuscularly or delivered to the interstitial space of a tissue. The compositions can also be administered into a tumor or lesion. Other modes of administration include oral and pulmonary administration, suppositories, and transdermal applications, needles, and gene guns or hyposprays. Dosage treatment may be a single dose schedule or a multiple dose schedule.

Methods for the *ex vivo* delivery and reimplantation of transformed cells into a subject are known in the art and described in e.g., International Publication No. WO 93/14778. Examples of cells useful in *ex vivo* applications include, for example, stem cells, particularly hematopoietic, lymph cells, macrophages, dendritic cells, or tumor cells.

Generally, delivery of nucleic acids for both *ex vivo* and *in vitro* applications can be accomplished by the following procedures, for example, dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei, all well known in the art.

EXAMPLES

The examples presented below are provided as a further guide to the practitioner of ordinary skill in the art, and are not to be construed as limiting the invention in any way.

Example 1

Cloning and Sequence Analysis of Myosin IXa

In order to identify novel transcripts in the human chromosomal region 15p22-23 which could be candidates for the Bardet-Biedl Sydrome gene that maps to this locus (BBS4; Bruford *et al.* (1997) *Genomics 41*:83-99) a BAC contig spanning this locus was constructed. A random clone sub-library was generated for each BAC. BAC clones forming a contig across the

chromosome 15q22-23 region were cleaved with CviJI under conditions that produced partial restriction fragments ranging in size from ~500 bp to ~4 kb. Fragments in the 1-2 kb range were isolated and cloned into the vector Perfectly Blunt™ (Novagen). For each BAC, 192 clones were randomly selected and sequenced using the ABI TAQ dye chemistry/system.

Comparison of these high throughput sample sequences originating from human chromosome 15 showed homology with 5 human EST clones (accession numbers AA287851, AA79085, AA235795, AA368273, HUMM9AA) and with myosin IXa (accession number L29148). Several clones were identified that showed significant homology to myr5 and human myosin IXb, and 100% identity to the entire 250 bp sequence known for human myosin IXa. Myosin IXa had previously been mapped to human chromosome 15q21-q25. Based on the information obtained from the comparisons of the myosin IXb sequence to the sequences of the inserts in these BAC sub-libraries, PCR primers were designed for Marathon™ 5' and 3' RACE™ reactions using Marathon™ prepared leukocyte, retina and testis cDNA (Clontech) as templates. Resulting fragments were cloned into pCRII (Invitrogen), sequenced and used for primer design for subsequent Marathon™ reactions. Nucleotide sequence for both strands of the partial cDNAs was obtained using the ABI TAQ dye terminator chemistry/system. Sequence comparison analysis was done using MASPAR and GeneWorks™ (Intelligenetics).

A sequence representing a transcript of 8473 bp was obtained. Human myosin IXa was mapped on the Stanford G3 radiation hybrid panel using oligos having homology to myosin IXa cDNA. Duplicate reactions consistently linked this region of myosin IXa to marker SHGC-31014 located on chromosome 15 in bin #50 confirming that the myosin IXa gene is located on human chromosome 15q21.

An ATG is present at base 243 and is in good context having an A at the -3 position and a G at +4 (Kozak *et al.* (1995)). This ATG initiates a single ORF that continues for 7643 bases to a stop signal (UGA) present at base 7886. Sequence analysis and exon mapping of the myo IXa transcript indicated that myosin IXa contains 42 exons and predicts a protein of 2548

amino acids with an estimated molecular mass of 283 kDa (Figure 1), a size similar to the myosin IXb gene product (Wirth *et al.* (1996)).

The myosin IXa gene spans over 185 kb in the human genome (data not shown) and produces a transcript whose predicted protein contains the domains described for class IX unconventional myosins. To determine the similarity between family members, the predicted amino acid sequence of human myosin IXa, myosin IXb, and rat myr5 were compared. The N-terminal extension of myosin IXa shares 40% identity with the other class IX myosins. Myosin IXa had 82% identity with myosin IXb and myr5 within the head domain that is conserved among known myosin classes, although myosin IXa also contained an 18 residue insertion absent in the other class IX family members. The position designated as the second flexible loop (20/50 kDa junction; which is proposed to contact actin (Rayment *et al.* (1993); Schroder *et al.* (1993)) and is the site of the second insertion in the head region of the class IX myosins, contained an additional 32 residues in myosin IXa in addition to having only 31% homology within the 120 residues shared with myosin IXb and myr5.

Within the neck region, myosin IXa contained two separate insertions of 33 and 18 residues compared to the other class IX family members. These insertions conform to the "IQ motif" and suggest that myosin IXa may bind 6 light chains of the clamodulin/EF hand superfamily (Mercer *et al.* 1991) instead of the 4 predicted to bind to myosin IXb and myr5.

The tail region of myosin IXa has 58% identity with the GAP domain found in myr5 and myosin IXb. However, the tail region located between the neck region and the GAP domain has numerous insertions not found in the other class IX family members that add an additional 327 residues compared to myr5 or 286 residues compared to myosin IXb.

Example 2

Expression of Myo IXa

To determine the expression profile of myosin IXa, human tissue Northern blots were hybridized with three different probes that represented the myosin IXa N-terminal extension plus a small portion of the conserved head

domain, the GAP domain and the 3' untranslated region. Briefly, human northern analysis was conducted on MTN Human, Human II and Human Brain II blots (Clontech). Probes corresponding to bases 9-1081, 6141-6831, and 7854-8304 were prepared using Amersham's Rediprime™ kit with ^{32}PαdCTP, hybridized in Express Hybridization solution (Clontech) as described by the manufacturer and exposed to BioMax™ film (Amersham).

A transcript of ~12.5 kb was visible in all tissues tested except liver. In addition, a transcript of ~8.5 kb was present in testis and placenta. To ascertain whether the 4 kb difference between the two testis messages involved alternative splicing and/or polyadenylation at the 3' region of the message, Marathon™ cDNA (Clontech) from testis, whole brain and cerebellum was amplified using a primer specific to the last exon (to bases 7704-7727) and the Marathon™ adaptor primer AP1. This PCR reaction produced a single band in all cases which corresponded to the product predicted from the cloned sequence. In addition, two brain cDNA libraries (frontal cortex and cerebellum (Stratagene)) were screened using the GAP domain probe. No evidence was found for either alternative splicing or polyadenylation at the 3' end of the myosin IXa transcript. Alternative splicing within and/or between the remaining identified exons was ruled out by conducting PCR amplification of whole brain, cerebellum, retina and testis cDNA with primers that spanned intron/exon junctions. No differences in product sizes were seen between those tissues expressing only the 12.5 kb transcript and testis. This result was confirmed by conducting multiple hybridizations to a frontal cortex cDNA library using probes representing each portion of the myosin IXA transcript. Thus, the 4 kb difference between the two transcript sizes present in testis was not due to alternative splicing between the identified exons nor due to alternative polyadenylation and most likely resides in the 5' untranslated region of the myosin IXa transcript. Therefore, the additional 4 kb present in the larger transcript is likely located in the 5' UTR.

However, at least one alternative splicing event does occur within the myosin IXa coding region. During the cloning, two distinct bands were generated from PCR reactions conducted on leukocyte cDNA with primers

specific to the head region. Sequence analysis of these bands indicated that a cryptic splice site exists at bases 375-380 and acts as a splice donor site, creating a truncated exon #2. Identical PCR reactions conducted on cDNA from testis and retina produced only the larger product. This data suggests that a second protein may be present in leukocytes, arising from an alternative splice within the second exon, which truncates the N-terminal extension and eliminates the ATTP binding domain of the myosin head region. No unique transcript for the predicted truncated protein was visible on Northern blots, presumably due to the decreased separation of molecules in the 12.0-12.5 kb size range.

Example 3

Developmental Expression of Myo IXa in a Mouse Model

A fragment specific to the mouse N-terminal extension of myosin IXa was obtained by conducting a PCR reaction on mouse genomic DNA using primers corresponding to bases 252.271 and bases 608-590 of the human myosin IXa sequence. This probe was prepared as described above and hybridized to mouse poly A^+ Northern blots prepared using standard methods from freshly dissected tissues. A single transcript of ~9 kb was detected. Myosin IXa was first visible in RNA from 11.5 d embryos, absent from 13.5 d embryos and reappeared in 15.5 d embryos. Myosin IXa was also present in RNA obtained from 13.5 d old limb buds and brain. Adult tissues also showed a myo IXa transcript of ~9 kb expressed in brain, heart, skeletal muscle, retina, leukocytes, lung, kidney, thymus and testis. No message was present in liver, a pattern identical with that seen in adult human tissues.

Example 4

Myo IXa functions as a GTPase activating protein

Myr5 acts as a GTP activating protein on members of the rho subfamily of GTP binding proteins (Reinhard *et al.* (1995) *EMBO J.* 14:697-704). We determined that myosin IXa is also a functional GAP by making an

expression construct that contained the predicted GAP domain (bases 6141 to 8035 of SEQ ID NO: 2) and conducting GAP assays with the small GTP binding proteins rhoA, ras, rac and Cdc42.

Example 5

Cloning and Sequence Analysis of CNGC-15 cDNA

A partial CNGC-15 cDNA, 1281 nucleotides in length was isolated from a human BAC contigs as described in Example 1. The cDNA sequence is shown in Figure 3. The ATG codon at nucleotide 36 g initiates an open reading frame that contiues through the rest of the cDNA. Comparison of the nucleotide to Genbank, EST and patent databases showed that this partial cDNA has 76.2% homology to a mouse ion channel BCGN-3 mRNA (Genbank accession number AF064874) and lesser homology to mouse and human ion channels. The longest stretch of 100% homology with sequences in the databases was 44 nucleotides.

THAT WHICH IS CLAIMED:

1. A native Myosin IXa polypeptide substantially free of other human proteins.

2. An active fragment or derivative of a Myosin IXa polypeptide.

3. A Myosin IXa polypeptide comprising an amino acid sequence selected from the group consisting of:

a) SEQ ID NO: 1;

b) residues 1-146 of SEQ ID NO: 1;

c) residues 147-719 of SEQ ID NO: 1;

d) residues 239-246 of SEQ ID NO: 1;

e) residues 720-835 of SEQ ID NO: 1;

f) residues 872-1166 of SEQ ID NO: 1;

g) residues 2074-2219 of SEQ ID NO: 1;

h) a polypeptide having at least about 61% homology to SEQ ID NO: 1;

i) a polypeptide at least about 95% homology to an amino acid sequence of b), c), d), e), f) or g); and

j) a polypeptide of at least about 90 contiguous residues of SEQ ID NO: 1.

4. A fusion protein comprising the Myosin IXa polypeptide of claim 3, or fragment thereof, and a polypeptide of interest.

5. A native CNGC-15 polypeptide substantially free of other human proteins.

6. An active fragment or derivative of a CNGC-15 polypeptide.

7. A CNGC-15 polypeptide comprising an amino acid sequence selected from the group consisting of:

a) a polypeptide encoded by SEQ ID NO: 3;

b) a polypeptide encoded by nucleotides 369-1307 of SEQ ID NO: 3;

c) a polypeptide having at least about 80% homology to the polypeptide encoded by nucleotides 369-1307 of SEQ ID NO: 3; and

d) a polypeptide of at least about 20 contiguous residues of the polypeptide encoded by nucleotides 369-1307 of SEQ ID NO: 3.

8. A fusion protein comprising the CNGC-15 polypeptide of claim 7, or fragment thereof, and a polypeptide of interest.

9. A nucleotide sequence encoding the fusion protein of claim 4.

10. A nucleotide sequence encoding the fusion protein of claim 8.

11. An isolated nucleotide sequence selected from the group consisting of:

a) a nucleotide sequence encoding a native Myosin IXa polypeptide;

b) a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 1;

c) the nucleotide sequence of SEQ ID NO: 2;

d) a nucleotide sequence comprising at least about 390 contiguous bases of SEQ ID NO: 2;

e) a nucleotide sequence comprising nucleotides 243-7085 of SEQ ID NO: 2;

f) a nucleotide sequence comprising nucleotides 243-680 of SEQ ID NO: 2;

g) a nucleotide sequence comprising nucleotides 683-2399 of SEQ ID NO: 2;

h) a nucleotide sequence comprising nucleotides 959-977 of SEQ ID NO: 2;

i) a nucleotide sequence comprising nucleotides 2402-2747 of SEQ ID NO: 2;

j) a nucleotide sequence comprising nucleotides 3158-3740 of SEQ ID NO: 2; and

k) a nucleotide sequence comprising nucleotides 6473-6899 of SEQ ID NO: 2;

12. An isolated nucleotide sequence selected from the group consisting of:

a) a nucleotide sequence encoding a native CNGC-15 polypeptide;

b) a nucleotide sequence encoding the polypeptide encoded by nucleotides 369-1307 of SEQ ID NO: 3;

c) the nucleotide sequence of SEQ ID NO: 3;

d) a nucleotide sequence comprising at least 45 contiguous nucleotides of the sequence of SEQ ID NO: 3; and

e) a nucleotide sequence that hybridizes under stringent conditions to a denatured DNA having a nucleotide sequence of a), b), c) or d).

13. An expression vector comprising the nucleotide sequence of claim 9 wherein said nucleotide sequence is operably linked to a promoter.

14. An expression vector comprising the nucleotide sequence of claim 10 wherein said nucleotide sequence is operably linked to a promoter.

15. An expression vector comprising the nucleotide sequence of claim 11 wherein said nucleotide sequence is operably linked to a promoter.

16. An expression vector comprising the nucleotide sequence of claim 12 wherein said nucleotide sequence is operably linked to a promoter.

17. A transformed cell having stably incorporated into its genome the expression vector of claim 13.

18. A transformed cell having stably incorporated into its genome the expression vector of claim 14.

19. A transformed cell having stably incorporated into its genome the expression vector of claim 15.

20. A transformed cell having stably incorporated into its genome the expression vector of claim 16.

21. A method for producing a Myosin IXa polypeptide or a derivative thereof, comprising:

a) providing a host cell stably transformed with an expression vector comprising a promoter operably linked to a nucleotide sequence encoding said Myosin IXa polypeptide or derivative thereof; and

b) culturing the host cell under conditions which allow expression of said polypeptide.

22. A method for producing a CNGC-15 polypeptide or a derivative thereof, comprising:

a) providing a host cell stably transformed with an expression vector comprising a promoter operably linked to a nucleotide sequence encoding said CNGC-15 polypeptide or derivative thereof; and

b) culturing the host cell under conditions which allow expression of said polypeptide.

23. A method for detecting an unconventional Myosin polynucleotide comprising:

a) providing a nucleic acid probe which hybridizes a nucleotide sequence of claim 11;

b) contacting the probe with a sample of polynucleotides under hybridizing conditions to form a duplex; and

c) detecting said duplexes.

24. A method for detecting a CNGC polynucleotide comprising:

a) providing a nucleic acid probe which hybridizes a nucleotide sequence of claim 12;

b) contacting the probe with a sample of polynucleotides under hybridizing conditions to form a duplex; and

c) detecting said duplexes.

25. An antibody specific for a CNGC-15 or Myosin IXa polypeptide.

26. A method for detecting a CNGC-15 polypeptide comprising:

a) providing an antibody that binds to a CNGC-15 polypeptide;

b) contacting the antibody to a sample under binding conditions to form a duplex; and

c) detecting said duplexes.

27. A method for detecting a Myosin IXa polypeptide comprising:

a) providing an antibody that binds to a Myosin IXa polypeptide;

b) contacting the antibody to a sample under binding conditions to form a duplex; and

c) detecting said duplexes.

28. A method for treating Bardet-Biedl Syndrome, Usher Syndrome, hearing loss, retinis pigmentosa, obesity, hypogonadism, sterility, polydactyly, brachydactyly, syndactyly, mental retardation, renal abnormalities, hypertension, diabetes and cardiovascular abnormalities,

comprising administering a therapeutically effective amount of a CNGC-15 polypeptide to a subject in need of such treatment.

29. A method for treating Bardet-Biedl Syndrome, Usher Syndrome, hearing loss, retinis pigmentosa, obesity, hypogonadism, sterility, polydactyly, brachydactyly, syndactyly, mental retardation, renal abnormalities, hypertension, diabetes and cardiovascular abnormalities, comprising administering a therapeutically effective amount of a Myosin-IXA polypeptide to a subject in need of such treatment.

30. A composition comprising a CNGC-15 polypeptide and a pharmaceutically acceptable carrier.

31. A composition comprising a Myosin IXa polypeptide and a pharmaceutically acceptable carrier.

MYOSIN IXa Polypeptide Sequence (SEQ ID NO:1)

```
MNINDGGRRRFEDNEHTLRIYPGAISEGTIYCPIPARKNSTAAEVIESLI      50
NKLHLDKTKCYVLAEVKEFGGEEWILNPTDCPVQQMMLWPRMALENRLSG     100
EDYRFLLREKNLDGSIHYGSLQSWLRVTEERRRMMERGFLPQPQQKDFDD     150
LCSLPDLNEKTLLENLRDRFKHEKIYTYVGSILIVINPFKFLPIYNPKYV     200
KMYDNHQLGKPEPHIYAVADVAYHAMLQRKKNQCIVISGESGSGKTQSTN     250
FLIHHLTALSQKGFASGVEQIILGAGPVLEAFGNAKTAHNNNSSRFGKFI     300
QVNYQETGTVLGAYVEKYLLEKSRLVYQEHNERNYHVFYYLLAGASEDER     350
SAFHLKQPEEYHYLNQITKKPLRQSWDDYCYDSEPDCFTVEGEDLRHDFE     400
RLQLAMEMVGFLPKTRRQIFSLLSAILHLGNICYKKKTYRDDSIDICNPE     450
VLPIVSELLEVKEEMLFEALVTRKTVTVGEKLILPYKLAEAVTVRNSMAK     500
SLYSALFDWIVFRINHALLNSKDLEHNTKTLSIGVLDIFGFEDYENNSFE     550
QFCINFANERLQHYFNQHIFKLEQEEYRTEGISWHNIDYIDNTCCINLIS     600
KKPTGLLHLLDEESNFPQATNQTLLDKFKHQHEDNSYIEFPAVMEPAFII     650
KHYAGKVKYGVKDFREKNTDHMRPDIVALLRSSKNAFISGMIGIDPVAVF     700
RWAILRAFFRAMVAFREAGKRNIHRKTGHDDTAPCAILKSMDSFSFLQHP     750
VHQRSLEILQRCKEEKYSITRKNPRTPLSDLQGMNALNEKNQHDTFDIAW     800
NGRTGIRQSRLSSGTSLLDKDGIFANSTSSKLLERAHGILTRNKNFKSKP     850
ALPKHLLEVNSLKHLTRLTLQDRITKSLLHLHKKKKPPSISAQFQASLSK     900
LMETLGQAEPYFVKCIRSNAEKLPLRFSDVLVLRQLRYTGMLETVQIRQS     950
GYSSKYSFQDFVSHFHVLLPRNIIPSKFNIQDFFRKINLNPDNYQVGKTM    1000
VFLKEQERQHLQDLLHQEVLRRIILLQRWFRVLLCRQHFLHLRQASVIIQ    1050
RFWRNYLNQKQVRDAAVQKDAFVMASAAALLQASWRAHLERQRYLELRAA    1100
AIVIQQKWRDYYRRRHMAAICIQARWKAYRESKRYQEQRKKIILLQSTCR    1150
GFRARQRFKALKEQRLRETKPEVGLVNIKGYGSLEIQGSDPSEWEDCSFD    1200
NRIKAIEECKSVIESNRISRESSVDCLKESPNKQQERAQSQSGVDLQEDV    1250
LVRERPRSLEDLHQKKVGRAKRESRRMRELEQAIFSLELLKVRSLGGISP    1300
SEDRRWSTELVPEGLQSPRGTPDSESSQGSLELLSYEESQKSKLESVISD    1350
EGDLQFPSPKISSSPKFDSRDNALSASNETSSAEHLKDGTMKEMVVCSSE    1400
SITCKPQLKDSFISNSLPTFFYIPQQDPLKTNSQLDTSIQRNKLLENEDT    1450
AGEALTLDINRETRRYHCSGKDQIVPSLNTESSNPVLKKLEKLNTEKEER    1500
QKQLQQQNEKEMMEQIRQQTDILEKERKAFKTIEKPRIGECLVAPSSYQS    1550
KQRVERPSSLLSLNTSNKGELNVLGSLSLKDAALAQKDSSSAHLPPKDRP    1600
VTVFFERKGSPCQSSTVKELSKTDRMGTQLNVACKLSNNRISKREHFRPT    1650
QSYSHNSDDLSREGNARPIFFTPKDNMSIPLVSKEALNSKNPQLHKEDEP    1700
AWKPVKLAGPGQRETSQRFSSVDEQAKLHKTMSQGEITKLAVRQKASDSD    1750
IRPQRAKMRFWAKGKQGEKKTTRVKPTTQSEVSPLFAGTDVIPAHQFPDE    1800
LAAYHPTPPLSPELPGSCRKEFKENKEPSPKAKRKRSVKISNVALDSMHW    1850
QNDSVQIIASVSDLKSMDEFLLKKVNDLDNEDSKKDTLVDVVFKKALKEF    1900
RQNIFSFYSSALAMDDGKSIRYKDLYALFEQILEKTMRLEQRDSLGESPV    1950
RVWVNTFKVFLDEYMNEFKTSDCTATKVPKTERKKRRKKETDLVEEHNGH    2000
IFKATQYSIPTYCEYCSSLIWIMDRASVCKLCKYACHKKCCLKTTAKCSK    2050
KYDPELSSRQFGVELSRLTSEDRTVPLVVEKLINYIEMHGLYTEGIYRKS    2100
GSTNKIKELRQGLDTDAESVNLDDYNIHVIASVFKQWLRDLPNPLMTFEL    2150
YEEFLRAMGLQERKETIRGVYSVIDQLSRTHLNTLERLIFHLVRIALQED    2200
TNRMSANALAIVFAPCILRCPDTTDPLQSVQDISKTTTCVELIVVEQMNK    2250
YKARLKDISSLEFAENKAKTRLSLIRRSMGKGRIRRGNYPGPSSPVVVRL    2300
PSVSDVSEETLTSEAAMETDITEQQQAAMQQEERVLTEQIENLQKEKEEL    2350
TFEMLVLEPRASDDETLESEASIGTADSSENLNMESEYAISEKSERSLAL    2400
SSLKTAGKSEPSSKLRKQLKKQQDSLDVVDSSVSSLCLSNTASSHGTRKL    2450
FQIYSKSPFYRAASGNEALGMEGPLGQTKFLEDKPQFISRGTFNPEKGKQ    2500
KLKNVKNSPQKTKETPEGTVMSGRRKTVDPDCTSNQQLALFGNNEFMV      2548
```

Figure 1

Human Myosin Ixa cDNA (SEQ ID NO:2)

GCGTCCGCTCGCCCGGACCCTGAGGCTGCTGGGCCCACCCTCCCGGAACC 50
GTCCGACCCTCGGTGGCCTCGGCTCGTTCTGCCATCTCCGGTCCTACCCT 100
GGGGCGGAGGGTGGAAGGCAGCTTCCGTCGAAGAGGAGGGGGCTGCGGTG 150
GCCACCGCGGCGGAGCCCGAGTTATTTTACCAAGAAAATGGTTTGCACGA 200
CTTTGAACATATACTATCCATGCTGATGGGACAGGATCCAATATGAATAT 250
AAATGATGGAGGAAGACGACGCTTTGAAGATAATGAACATACATTACGGA 300
TATATCCTGGGGCTATTTCAGAAGGGACAATCTACTGTCCGATTCCTGCC 350
AGAAAAAACTCCACAGCTGCTGAGGTGATTGAGTCTCTTATAAACAAACT 400
TCATCTTGACAAAACAAAATGTTACGTTCTAGCAGAGGTAAAGGAATTTG 450
GTGGAGAAGAATGGATTCTCAATCCAACAGATTGTCCAGTTCAGCAAATG 500
ATGCTGTGGCCCCGAATGGCTCTGGAAAATCGCTTAAGTGGAGAAGACTA 550
CCGCTTCCTTCTGAGAGAGAAAAACCTTGATGGATCAATCCATTATGGTA 600
GCCTGCAGTCATGGCTACGGGTAACAGAAGAACGTCGCAGGATGATGGAA 650
CGGGGTTTTCTTCCACAGCCTCAACAGAAAGACTTTGATGATTTATGTAG 700
TTTACCTGATTTGAATGAGAAAACTCTCTTAGAAAACCTACGAGATCGCT 750
TTAAGCATGAAAAAATTTATACCTATGTTGGCAGTATTCTAATAGTTATT 800
AACCCATTCAAGTTTCTTCCTATTTATAACCCCAAATATGTCAAAATGTA 850
TGATAACCACCAACTGGGAAAACCTGAGCCCCACATTTATGCTGTGGCTG 900
ATGTAGCTTATCATGCCATGCTTCAGCGCAAAAAGAATCAGTGCATCGTG 950
ATTTCAGGAGAGAGTGGTTCTGGGAAGACTCAAAGCACAAACTTTCTTAT 1000
TCACCACCTTACTGCTCTCAGTCAGAAAGGATTTGCCAGTGGAGTAGAAC 1050
AGATTATTCTTGGAGCTGGACCAGTACTTGAGGCCTTTGGAAATGCAAAG 1100
ACAGCTCATAATAACAATTCAAGTCGTTTTGGGAAGTTTATTCAAGTAAA 1150
TTACCAGGAAACAGGCACTGTACTTGGTGCCTATGTTGAAAAATATCTAC 1200
TGGAGAAGTCCAGACTCGTTTATCAGGAGCATAATGAACGGAACTATCAT 1250
GTATTCTATTACCTCCTGGCAGGAGCAAGTGAAGATGAGAGATCAGCATT 1300
CCATCTTAAGCAACCAGAGGAATATCATTATCTCAATCAGATAACAAAGA 1350
AACCCCTCAGACAGAGCTGGGATGATTATTGCTATGACTCTGAGCCGGAT 1400
TGCTTCACGGTGGAAGGAGAAGATTTGAGACATGACTTTGAGCGCCTACA 1450
ACTTGCCATGGAAATGGTAGGATTTCTTCCCAAGACACGAAGACAGATTT 1500
TCTCTCTTCTCTCAGCCATACTACATTTGGGTAATATCTGTTACAAAAAG 1550
AAGACATACCGGGATGACTCCATTGATATCTGTAATCCTGAAGTTCTGCC 1600
TATTGTCTCAGAATTATTAGAGGTTAAAGAAGAGATGCTATTTGAAGCAT 1650
TAGTTACAAGGAAGACGGTGACAGTGGGAGAAAAGCTTATTTTGCCATAC 1700
AAGTTGGCAGAGGCTGTGACAGTGAGGAACTCCATGGCTAAGTCTCTGTA 1750
TAGTGCCCTGTTTGACTGGATAGTTTTTCGAATTAATCATGCACTTCTGA 1800
ATAGTAAAGATTTAGAGCATAATACCAAGACATTGTCTATTGGTGTTCTT 1850
GATATTTTTGGGTTTGAAGATTATGAAAATAACAGCTTTGAACAGTTCTG 1900
TATTAATTTTGCTAATGAACGTTTACAGCACTACTTTAATCAGCATATCT 1950
TTAAATTGGAACAAGAGGAATATAGAACTGAAGGTATCAGCTGGCACAAC 2000
ATAGATTACATTGATAATACCTGCTGCATAAATCTTATTAGCAAAAAACC 2050
AACAGGACTGCTTCATCTTTTGGATGAAGAAAGCAACTTTCCACAGGCTA 2100
CAAATCAAACATTGCTAGACAAGTTTAAGCATCAACATGAAGATAATTCT 2150

Figure 2

TACATCGAATTTCCAGCCGTGATGGAGCCTGCTTTCATTATAAAACATTA 2200
TGCTGGAAAAGTAAAATATGGGGTAAAGGATTTCCGGGAAAAAAATACAG 2250
ATCATATGCGCCCAGACATTGTAGCTCTTCTGAGAAGCAGCAAGAATGCA 2300
TTTATCTCTGGGATGATTGGAATTGATCCTGTAGCTGTTTTCCGATGGGC 2350
AATTCTCCGAGCTTTTTTCAGAGCCATGGTTGCTTTCAGGGAAGCTGGGA 2400
AAAGAAACATTCACAGAAAAACTGGACATGATGATACAGCGCCATGTGCA 2450
ATTTTGAAAAGTATGGATAGTTTTAGCTTTCTCCAACACCCAGTCCACCA 2500
GAGGAGCTTAGAGATTCTGCAGAGATGCAAGGAAGAGAAGTACAGTATAA 2550
CCCGGAAAAATCCCAGAACACCTCTTTCTGATCTCCAGGGCATGAATGCT 2600
CTAAATGAAAAAAACCAACATGATACATTTGATATTGCCTGGAATGGCAG 2650
AACTGGGATTCGCCAGAGCAGACTATCAAGTGGCACCTCCTTGCTTGATA 2700
AAGATGGAATATTTGCTAATTCAACTAGCAGCAAACTCCTGGAGAGAGCC 2750
CATGGAATTCTCACGAGAAACAAAAATTTCAAATCCAAGCCTGCCCTTCC 2800
AAAGCACTTGCTAGAAGTAAATTCTTTAAAGCACCTGACAAGACTGACAC 2850
TACAAGATCGCATTACCAAGTCTCTTCTTCATTTACACAAGAAGAAAAAA 2900
CCTCCCAGCATCAGTGCCCAGTTTCAGGCATCATTAAGCAAGCTAATGGA 2950
AACACTTGGTCAAGCAGAACCATATTTTGTAAAATGCATTCGCTCTAATG 3000
CTGAAAAGCTGCCCTTAAGGTTCAGTGATGTCTTGGTACTTAGACAGCTT 3050
CGATACACCGGGATGCTGGAAACAGTTCAAATTCGCCAATCAGGATACAG 3100
CTCCAAATATTCTTTCCAGGATTTTGTGAGCCACTTCCATGTACTTCTTC 3150
CCCGAAATATTATTCCATCCAAATTTAACATTCAGGATTTCTTCAGGAAA 3200
ATAAATCTTAATCCAGATAATTATCAAGTTGGAAAAACCATGGTCTTTCT 3250
AAAGGAGCAGGAACGACAGCACTTACAAGATCTGCTTCACCAAGAGGTGC 3300
TCCGCAGAATCATATTGTTGCAGCGATGGTTCAGGGTCTTGCTGTGTAGG 3350
CAGCATTTCCTCCATCTGAGACAAGCATCTGTTATTATCCAGAGATTCTG 3400
GAGGAATTACCTAAATCAGAAGCAAGTCAGAGATGCAGCTGTGCAGAAGG 3450
ATGCTTTTGTTATGGCTAGTGCAGCTGCTCTTCTCCAAGCTTCCTGGCGT 3500
GCTCACTTAGAGAGGCAGCGGTACTTGGAGTTACGGGCTGCAGCCATCGT 3550
TATCCAGCAGAAATGGAGAGATTACTATAGGCGCAGGCACATGGCTGCTA 3600
TTTGCATACAAGCAAGATGGAAAGCCTACAGGGAAAGTAAAAGGTACCAA 3650
GAACAAAGGAAAAAAATTATCCTTTTGCAATCAACATGTAGAGGATTCAG 3700
AGCAAGACAAAGATTTAAAGCTTTAAAAGAACAAAGGCTAAGAGAAACAA 3750
AGCCAGAAGTTGGATTGGTGAATATTAAGGGATATGGATCTCTGGAAATT 3800
CAGGGTTCAGACCCTTCAGAATGGGAGGATTGTTCTTTTGACAACAGAAT 3850
AAAAGCCATAGAGGAATGTAAATCTGTAATAGAGAGTAATCGAATTAGCC 3900
GTGAAAGTTCAGTGGACTGCTTGAAGGAGTCACCAAACAAGCAGCAGGAG 3950
AGAGCCCAAAGCCAGAGTGGTGTGGACTTGCAGGAAGATGTGCTTGTAAG 4000
AGAGAGACCCAGGTCCTTGGAGGATCTCCATCAGAAAAAAGTAGGCCGGG 4050
CTAAGAGAGAAAGTAGGAGAATGAGAGAACTAGAGCAAGCTATATTTAGC 4100
TTAGAATTGCTGAAAGTTCGTTCTCTTGGTGGTATTTCTCCTTCAGAGGA 4150
TCGCAGATGGTCTACAGAATTGGTGCCTGAAGGCCTTCAGTCTCCACGGG 4200
GTACACCTGATAGTGAGAGCTCTCAAGGAAGCTTGGAACTTCTGAGCTAT 4250
GAGGAAAGCCAAAAGAGCAAACTAGAGTCTGTCATTTCAGATGAAGGAGA 4300
CTTGCAGTTTCCATCACCTAAGATATCCAGCAGTCCAAAATTTGATTCAC 4350
GGGACAATGCCCTCAGTGCCTCAAATGAGACTAGCAGTGCAGAGCATTTG 4400

Figure 2, con't

AAGGATGGAACTATGAAGGAAATGGTGGTCTGCAGTTCTGAGTCTATTAC 4450
CTGTAAACCACAGCTGAAAGACTCCTTCATTTCAAATAGTCTACCTACTT 4500
TTTTTTATATCCCCCAACAAGACCCACTGAAAACAAATTCCCAACTAGAC 4550
ACAAGTATCCAAAGAAACAAACTATTGGAAAATGAAGACACAGCGGGGGA 4600
AGCTCTTACTTTGGATATCAACAGGGAAACTAGAAGGTATCACTGCTCAG 4650
GAAAAGATCAGATTGTTCCTTCTTTGAATACAGAGTCTTCTAATCCTGTG 4700
CTTAAGAAGTTAGAAAAGCTAAACACTGAGAAGGAAGAAAGGCAAAAACA 4750
GTTGCAGCAACAGAATGAAAAAGAGATGATGGAACAGATTCGCCAGCAAA 4800
CAGATATTTTAGAGAAGGAGCGCAAAGCCTTCAAGACAATTGAAAAGCCA 4850
AGAATTGGAGAGTGTTTGGTGGCACCATCTTCCTATCAGTCAAAGCAAAG 4900
AGTAGAGAGGCCATCCTCTCTCCTCAGCTTAAATACCTCAAATAAGGGAG 4950
AACTTAATGTACTGGGGTCCCTATCATTAAAAGATGCAGCTCTTGCCCAA 5000
AAAGACAGTTCCTCTGCTCACTTACCCCCAAAGGACCGACCTGTCACCGT 5050
GTTCTTTGAAAGAAAAGGAAGTCCATGCCAATCTAGTACTGTCAAGGAAT 5100
TATCCAAGACAGACAGAATGGGCACCCAGCTGAATGTAGCCTGTAAACTC 5150
TCAAATAATCGCATTTCAAAAAGAGAACACTTTAGGCCAACTCAGTCTTA 5200
CAGCCACAATTCTGATGACCTTTCCAGAGAGGGAAATGCTAGGCCCATTT 5250
TCTTCACTCCAAAGGATAATATGAGTATTCCCCTTGTCAGCAAAGAAGCC 5300
TTAAACAGTAAAAATCCTCAACTCCATAAAGAAGATGAACCAGCATGGAA 5350
ACCTGTGAAGTTAGCTGGGCCAGGCCAAAGAGAGACATCACAGCGATTTT 5400
CGTCAGTTGATGAACAAGCAAAACTTCATAAGACTATGTCTCAAGGAGAG 5450
ATTACCAAGTTGGCAGTGAGACAGAAGGCTTCAGATTCAGATATAAGACC 5500
TCAGAGAGCTAAGATGAGATTCTGGGCCAAAGGGAAACAAGGGGAGAAGA 5550
AGACTACCAGAGTGAAACCTACTACCCAGTCAGAGGTTTCGCCACTCTTT 5600
GCAGGCACAGATGTGATTCCAGCTCATCAGTTTCCAGATGAATTAGCTGC 5650
ATATCACCCAACACCTCCTTTGAGCCCAGAACTGCCCGGCAGTTGCCGGA 5700
AGGAATTCAAAGAGAACAAAGAACCTTCTCCAAAGGCTAAGCGCAAGCGA 5750
AGTGTGAAGATTAGCAACGTGGCTTTGGATTCTATGCATTGGCAAAATGA 5800
CTCTGTCCAGATCATAGCAAGTGTCAGTGATTTAAAAAGCATGGATGAAT 5850
TTCTTCTGAAAAAGGTGAATGACCTAGATAATGAAGACAGCAAGAAGGAT 5900
ACACTAGTGGATGTTGTATTTAAAAAAGCCCTGAAGGAATTTCGGCAGAA 5950
TATCTTCAGCTTTTATTCATCTGCATTGGCGATGGATGATGGGAAAAGCA 6000
TACGGTATAAAGACCTCTATGCACTATTTGAACAGATTCTGGAAAAGACG 6050
ATGAGGCTTGAGCAGCGTGATTCACTGGGTGAATCTCCAGTGAGAGTTTG 6100
GGTCAACACTTTTAAAGTGTTTTTAGATGAATATATGAATGAATTCAAGA 6150
CTTCAGATTGCACAGCCACAAAGGTGCCAAAAACAGAAAGAAAGAAAAGA 6200
AGGAAAAAGGAAACTGATTTGGTGGAAGAACACAATGGTCACATCTTTAA 6250
AGCCACCCAATATAGCATCCCTACATACTGTGAATACTGTTCTTCTTTGA 6300
TATGGATAATGGACCGAGCCTCTGTTTGCAAATTATGCAAGTATGCTTGC 6350
CATAAGAAGTGCTGTCTGAAAACCACAGCCAAGTGCTCTAAAAAGTATGA 6400
TCCAGAGCTGTCATCTCGACAATTTGGGGTTGAACTGTCCCGTTTGACCA 6450
GTGAAGACCGAACTGTTCCTTTAGTAGTGGAAAAGCTCATAAACTACATT 6500
GAAATGCATGGACTGTATACAGAAGGTATTTATCGAAAGTCTGGTTCGAC 6550
TAATAAAATCAAGGAGCTTCGGCAGGGTCTAGATACAGATGCTGAGAGTG 6600
TAAATCTAGATGACTATAACATACACGTCATTGCAAGTGTATTCAAACAA 6650

Figure 2, con't

```
TGGCTTCGAGATTTGCCCAATCCTCTCATGACCTTTGAACTCTATGAGGA      6700
ATTTCTTCGAGCTATGGGCCTTCAGGAGAGGAAGGAGACAATCCGTGGTG      6750
TATACTCTGTGATTGATCAACTCTCCCGAACTCATCTCAATACACTGGAA      6800
CGCCTCATCTTTCATCTAGTCAGGATTGCTCTGCAGGAAGACACTAATCG      6850
AATGTCTGCTAATGCTTTGGCCATTGTGTTTGCGCCCTGCATTCTCCGCT      6900
GCCCTGACACCACTGACCCACTACAAAGTGTACAGGACATCAGTAAGACT      6950
ACCACTTGTGTGGAACTGATTGTTGTGGAACAAATGAATAAATACAAGGC      7000
TCGTCTCAAAGATATCAGTAGCTTGGAATTTGCTGAGAATAAGGCAAAGA      7050
CCAGGTTGTCACTGATTCGTAGATCAATGGGAAAGGGGCGTATTCGTCGA      7100
GGAAACTATCCAGGTCCATCGTCTCCTGTTGTAGTTCGGTTGCCTTCTGT      7150
GTCTGATGTCTCAGAGGAGACCTTGACTAGTGAGGCAGCCATGGAGACTG      7200
ACATCACAGAACAGCAGCAAGCAGCTATGCAGCAGGAGGAGAGAGTACTG      7250
ACTGAGCAGATTGAGAACCTACAGAAGGAGAAGGAGGAGCTAACATTTGA      7300
GATGCTTGTACTGGAACCCCGTGCCTCTGATGATGAAACCCTTGAGTCTG      7350
AGGCCTCCATTGGGACTGCTGATAGCTCAGAGAATTTGAATATGGAGTCT      7400
GAATATGCTATCTCTGAGAAATCAGAAAGAAGCTTAGCCCTTAGCTCCCT      7450
GAAGACAGCTGGCAAGTCTGAACCTTCCAGCAAGTTGCGAAAGCAACTTA      7500
AAAAGCAGCAAGACTCTTTAGATGTCGTGGACTCTTCGGTCTCCTCTTTA      7550
TGTCTGTCTAACACGGCATCATCTCATGGGACCAGAAAACTATTTCAGAT      7600
TTATTCCAAATCTCCATTCTACCGAGCTGCCTCAGGTAATGAGGCCCTGG      7650
GAATGGAAGGACCATTGGGCCAGACCAAATTCCTGGAAGACAAGCCTCAG      7700
TTCATCAGCAGAGGAACCTTCAACCCGGAAAAGGGCAAACAAAAATTAAA      7750
GAATGTGAAAAACTCACCTCAGAAAACCAAAGAGACCCCAGAGGGGACAG      7800
TCATGTCTGGCCGCAGAAAAACTGTGGACCCAGACTGCACCTCCAACCAA      7850
CAGCTAGCACTCTTTGGAAATAATGAATTTATGGTCTGAACCGGCAGATG      7900
TGTGTCCCTCCGTGGCTACAGAGTGGTAAACAAATCTCACCTTTGGGGCT      7950
GCGTTTCATCACCTCGTCCACAATAGTCAATCCTAATTGTGGTCCTGCCT      8000
CTTTTCTAAGCATATGGCTAAGACTGTATGTGCTGAATTCCTGGGCCTCC      8050
TGCAGAAGCAGAAAGCCTGCTGGGGATGGTGCCAGCTGTGCCTTGGCTGT      8100
TGTATTTGAATTGAGATTTTTACTATACAAAGCCACCTAGGGCCTGGGGA      8150
TTTGGGTCAGTTGTAGTTGCCTCTCCCCCACCCTCTTTTCCCTTCCCAAA      8200
GGTGGGTGTTGAACTAGGGGGGATATTGCTGTCCTGAGGGACCCTCTCAT      8250
TTCTGACATTTGAAGAAAACGTATAAATCTTTCTTAACCGTGAAAGCAAA      8300
AGCCTTTGGGTTTATTTTGGGATAGTTAGGAGCTAGGGTAGAATATAATT      8350
TTTTTCCAAAAACTTACTTACAAACAAAAAGCCTAATCCCTCTATTTTAA      8400
GATTTCTGAAAAAACACTCCATGTTATATTCTGGGGAAAGCAAAAACAAA      8450
AAAAAAAAAAAAAAAAAAAAAA                                  8473
```

Figure 2, con't

CNGC-15 Partial cDNA (SEQ ID NO:3)

```
GAATTCGGGCTTCCATCCTTAATAGGAACTCAATNNTAGGGCTNGGGCGG     50
CCGCCCGGGCAGGTGCGCGAACAGGAGAGGGTCAAGTCGGCCGGATTTTG   100
GATTATCCACCCCTACAGTGACTTCAGATTTTACTGGGACCTGACCATGC   150
TGCTGCTGATGGTGGGAAACCTGATTATCATTCCTGTGGGCATCACCTTC   200
TTCAAGGATGAGAACACCACACCCTGGATTGTCTTCAATGTGGTGTCAGA   250
CACATTCTTCCTCATCGACTTGGTCTTCAACTTCCGCACAGGGATCGTGG   300
TGGAGGACAACACAGAGATCATCCTGGACCCGCAGCGGATTAAAATGAAG   350
TACCTGAAAAGCTGGTTCATGGTAGATTTCATTTCCTCCATCCCCCGTGG   400
AAAACATCTTCCTCATTGTGGAGACAGGCATCGACTCGGAGGTCTACAAG   450
ACTGCCCGGGCCCTGCGCATTGTCCGCTTCACGAAGATCCTCAGCCTCTT   500
ACGCCTGTTACGCCTCTCCCGCCTCATTCGATATATTCACCAGTGGGAAG   550
AGATCTTCCACATGACCTACGACCTGGCCAGCGCCGTGGTGCGCATCGTG   600
AACCTCATCGGCATGATGCTCCTGCTCTGCCACTGGGACGGCTGCCTGCA   650
GTTCCTGGTACCCATGCTACAGGACTTCCCTGACGACTGCTGGGTGTCCA   700
TCAACAACATGGTGAACAACTCCTGGGGGAAGCAGTACTCCTACGCGCTC   750
TTCAAGGCCATGAGCCACATGCTGTGCATCGGCTACGGGCGGCAGGCGCC   800
CGTGGGCATCTCCGACGTCTGGCTCACCATGCTCAGCATGATCGTGGGTG   850
CCACCTGCTACGCCATGTTCATTGGCCACGCCACTGCCCTCATCCAGTCC   900
CTGGACTCCTCCCGGCGCCAGTACCAGGAAAAGTACAAGCAGGTGGAGCA   950
GTACATGTCCTTTCACAAGCTCCCGCCCGACACCCGGCAGCGCATCCACG  1000
ACTACTACGAGCACCGCTACCAGGGCAAGATGTTCGACGAGGAGAGCACC  1050
CTGGGCGAGCTAAGCGAGCCCCTGCGGGAGGAGATCATCAACTTTAACTG  1100
TCGGAAGCTGGTGGCCTCCATGCCACTGTTTGCCAATGCGGACCCCAACT  1150
TCGTGACGTCCATGCTGACCAAGCTGCGTTTCGAGGTCTTCCAGCCTGGG  1200
GACTACATCATCCGGGAAGGCACCATTGGCAAGAAGATGTACTTCATCCA  1250
GCATGGCGTGGTCAGCGTGCTCACCAAGGGCAACAAGGAGACCAAGAAGC  1300
CGAATTC                                              1307
```

Figure 3

INTERNATIONAL SEARCH REPORT

Internatic Application No
PCT/US 98/21971

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C07K14/47 C07K14/705 C12N15/62 C07K16/28
G01N33/50 C12Q1/68 A61K38/17

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K G01N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BEMENT WM ET AL.: "Identification and overlapping expression of multiple unconventional myosin genes in vertebrate cell types" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 91, July 1994, pages 6549-6553, XP002097350 WASHINGTON US cited in the application see figures 1,2	1,2,11
X	& BEMENT WM: 'Homo sapiens myosin mRNA, partial cds' EMBL database entry HSM9AA Accession number L29148, 02-Mar-1994 see sequence	11

-/--

[X] Further documents are listed in the continuation of box C. [ ] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 March 1999	07/04/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Espen, J

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	NATIONAL CANCER INSTITUTE, CANCER GENOME ANATOMY PROJECT (CGAP): "Homo sapiens cDNA clone similar to TR:G1147783, Myosin-IXB" EMEST DATABASE ENTRY HS1183475, ACCESSION NUMBER AA279085,3 April 1997, XP002097351 see sequence ---	11
X	HILLIER L ET AL: "Homo sapiens cDNA clone 726913" EMEST DATABASE ENTRY HS1202408, ACCESSION NUMBER AA403319,1 May 1997, XP002097352 see sequence ---	11
X	NATIONAL CANCER INSTITUTE, CANCER GENOME ANATOMY PROJECT (CGAP): "Homo sapiens cDNA clone similar to TR:G1147783, Myosin-IXB" EMEST DATABASE ENTRY HS1191094, ACCESSION NUMBER AA287851, XP002097353 see sequence ---	11
X	HILLIER L ET AL: "Homo sapiens cDNA clone 773282 5' similar to TR:G639999 Myosin Heavy Chain" EMEST DATABASE ENTRY HS1224967, ACCESSION NUMBER AA425434,24 May 1997, XP002097354 see sequence ---	11
X	HILLIER L ET AL: "Homo sapiens cDNA clone 727977 3' EST" EMEST DATABASE ENTRY HS1198948, ACCESSION NUMER AA398548,28 April 1997, XP002097355 see sequence ---	11
Y	HASSON, TAMA ET AL: "Vertebrate unconventional myosins" J. BIOL. CHEM. (1996), 271(28), 16431-16434 CODEN: JBCHA3;ISSN: 0021-9258, XP002097356 see table 1 ---	2
Y	POST, P. L. (1) ET AL: "Human myosin IX: A novel unconventional myosin with a chimaerin-like RHO/RAC GAP homology domain in its tail." MOLECULAR BIOLOGY OF THE CELL, (1995) VOL. 6, NO. SUPPL., PP. 144A. MEETING INFO.: THIRTY-FIFTH ANNUAL MEETING OF THE AMERICAN SOCIETY FOR CELL BIOLOGY WASHINGTON, D.C., USA DECEMBER 9-13, 1995 ISSN: 1059-1524., XP002097357 see abstract --- -/--	2

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INTERNATIONAL SEARCH REPORT

Internati. Application No
PCT/US 98/21971

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	HASSON T ET AL: "Mapping of unconventional myosins in mouse and human." GENOMICS, (1996 SEP 15) 36 (3) 431-9. JOURNAL CODE: GEN. ISSN: 0888-7543., XP002097358 United States see abstract; tables 1,2	2
A	WELLS C ET AL: "Myogenic cells express multiple myosin isoforms." JOURNAL OF MUSCLE RESEARCH AND CELL MOTILITY, (1997 OCT) 18 (5) 501-15. JOURNAL CODE: HSN. ISSN: 0142-4319., XP002097359 ENGLAND: United Kingdom	
A	WEIL D ET AL: "Human myosin VIIA responsible for the Usher 1B syndrome: a predicted membrane-associated motor protein expressed in developing sensory epithelia." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1996 APR 16) 93 (8) 3232-7. JOURNAL CODE: PV3. ISSN: 0027-8424., XP002097360 United States	
X	HILLIER ET AL: "Homo sapiens cDNA clone 289005 5' similar to PIR:S52072 DmCNGC protein-fruit fly" EMEST DATABASE ENTRY HSN72770, ACCESSION NUMBER N72770,20 March 1996, XP002097361 see sequence	12
P,X	SANTORO B ET AL: "Identification of a gene encoding a hyperpolarization-activated pacemaker channel of brain" CELL, vol. 93, 29 May 1998, pages 717-729, XP002097362 NA US see abstract	5,6,12

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INTERNATIONAL SEARCH REPORT

Internal application No.
PCT/US 98/21971

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claims 28,29
are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See additional sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [X] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-4,9,11,21,23,27,29,31; in part 25

   Subject-matter relating to myosin IXa

2. Claims: 5-8,10,12-20,22,24,26,28,30; in part 25

   Subject-matter relating to CNGC-15